U.S. DEPARTMENT OF COMMERCE
National Technical Information Service

PB-260 811

Proposed Water Quality Information. Volume II

Environmental Protection Agency, Washington, D C

Oct 73

BIBLIOGRAPHIC DATA SHEET	1. Report No.	2.	3. Recipient's Accession No. PB-260 811

4. Title and Subtitle Proposed Water Quality Information. Volume II.	5. Report Date Oct 73 6.
7. Author(s)	8. Performing Organization Rept. No.
9. Performing Organization Name and Address Environmental Protection Agency, Washington, D. C.	10. Project/Task/Work Unit No. 11. Contract/Grant No.
12. Sponsoring Organization Name and Address	13. Type of Report & Period Covered 14.

15. Supplementary Notes

16. Abstracts

This report is Volume II of a two-volume publication. It contains information on what is found regionally in the Nation's water; on the natural and man-made sources of polluting; on the measurement and classification of water quality; and on the identification of pollutants suitable for maximum daily load measurement. In fact the report primarily discusses the techniques used in water analysis.

17. Key Words and Document Analysis. 17a. Descriptors

*Water quality,
*United States,
*Water analysis,

Sources,
pH,
Halides,
Biological oxygen demand,
Dissolved gases,
Oxygen,
Trace elements,
Metals,
Nitrogen,
Phosphorus,
Sulfates,
Sediments,
Plankton.

17b. Identifiers/Open-Ended Terms

*Water pollution detection,
Path of pollutants,
Chemical oxygen demand.

17c. COSATI Field/Group 7D

18. Availability Statement National Technical Information Service Springfield, Va. 22161	19. Security Class (This Report) UNCLASSIFIED	21. No. of Pages 174p
	20. Security Class (This Page) UNCLASSIFIED	22. Price 6.75/3.00

338047

PB 260 811

PROPOSED WATER QUALITY INFORMATION

Volume II

OCTOBER 1973

U.S. ENVIRONMENTAL PROTECTION AGENCY
Washington, D.C. 20460

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U. S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

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FOREWORD

The Federal Water Pollution Control Act Amendments require the Administrator of the U.S. Environmental Protection Agency to publish both criteria for water quality and information for the restoration and maintenance of aquatic integrity, and the measurement and classification of water [Section 304a(a)1 and 2, P.L. 92-500].

Volume I of this two volume series contains the criteria for water quality for the protection of human health and for the protection and propagation of desirable species of aquatic biota. Volume II of the series contains information on the maintenance and restoration, measurement, and the classification of waters. Also those pollutants suitable for maximum daily load calculations are identified.

Both Volumes I and II are published as proposed documents with a 180 day period provided for public comment.

Russell E. Train

Russell E. Train
Administrator

Volume II
Proposed
Information for Water Quality
Environmental Protection Agency

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Information for Water Quality

I. Legislative Basis

Section 304(a)(2) of the "Federal Water Pollution Control Act Amendments of 1972" (86 Stat. 816; 33 U.S.C. 1314 1972), hereinafter referred to as the "Act", provides that the Administrator (EPA) shall, within one year of enactment, (by Oct. 18, 1973), publish information: (A) on the factors necessary to restore and maintain the chemical, physical and biological integrity of the aquatic environment; (B) on the factors necessary for the protection and propagation of shellfish, fish and wildlife for all classes and categories of receiving waters and to allow recreational activities in and on the water; (C) on the measurement and classification of water quality; and (D) on the identification of pollutants suitable for maximum daily load measurement correlated with the achievement of water quality objectives. The information shall reflect the latest scientific knowledge.

II. Introduction

The National Water Quality Standards Program was initiated with the passage of the Water Quality Act of 1965, Sec 10(c). The Water Quality Standards are comprised of use designations for each water body or portion thereof, water quality criteria to support the use designations, and implementation plans for scheduling the construction of the necessary treatment facilities. The designations by water uses are protection and propagation of fish, aquatic life and wildlife (fresh water and marine), water for public water supplies, recreational, agricultural, and industrial waters. The Water Quality Standards prior to enactment of FWPCA Amendments of 1972 were applicable to only interstate waters and their tributaries. The Act extends the coverage to include all intrastate waters and the state standards have since been, or are in the process of being revised accordingly.

The objective of the Act is to restore and maintain the chemical, physical and biological integrity of the Nation's waters. The National goal, Sec 101(a)(1), is to eliminate the discharge of pollutants into navigable waters by 1985, with an interim goal, Sec 101(a)(2), being to attain by July 1983, water quality which provides for the protection and propagation of fish, shellfish and wildlife and for recreation in and on the Nation's water.

→ This report

This Information for Water Quality is Volume II of a two-volume publication. It contains information on what is found regionally in the Nation's water; on the measurement and classification of water quality; and on the identification of pollutants suitable for maximum daily load measurement. Volume I, under separate cover, is the Criteria document. The Criteria for Water Quality compiled within Volume I consists of prescribed limits of acceptability for pollutants, each followed immediately by supporting scientific rationale. The Information for Water Quality is provided to assist the user of Volume I in his application of the Water Quality Criteria.

→ In fact
the report primarily
discusses the techniques
used in water analysis.

III. Factors for Consideration for the Maintenance and Restoration of Integrity of Water and for the Protection and Propagation of Aquatic Life and Wildlife

Restoration and maintenance of aquatic integrity requires the identification and quantification of the elements or components acting on the environment and an understanding of the effect each has on the aquatic environment. Presented in Table 1 are the ranges and mean concentrations of inorganic pollutants found in the major freshwater basins in the United States. Data are arranged by basins, and give the source of pollutants and dates data were collected.

on the ~~[illegible] of [illegible]~~ the
natural and man-made sources
of pollution

	Sources		Concentrations in U.S. Waters	
Agent	Natural	Man-Made	Basin Areas	Concentrations (mg/l)
Aluminum	Weathering of rock	Industrial waste, mine drainage, and wash water from water treatment plants	New Jersey surface water	1968
			1) spring flow	0.09-0.33
			2) summer flow	0.08-0.31
			Northeast Basin	Mean Positive: 0.028 1962-67
			North Atlantic Basin	0.022
			Southeast Basin	0.117
			Tennessee R. Basin	0.030
			Ohio R. Basin	0.141
			Lake Erie Basin	0.056
			Upper Mississippi R. Basin	0.018
			Western Great Lakes Basin	0.017
			Missouri R. Basin	0.213
			Lower Mississippi R. Basin	0.068
			Colorado R. Basin	0.050
			Western Gulf Basin	0.333
			Pacific Northwest Basin	0.030
			California Basin	0.063
			Great Basin	0.015
			Alaska Basin	0.011
Antimony		Industrial effluents, but is rapidly removed by precipitation and adsorption	Principal rivers of U.S.	None detected 1958-59

	Sources		Concentrations in U.S. Waters		
Agent	Natural	Man-Made	Basin Areas	Concentrations (mg/l)	
Arsenic	Weathering of rocks	Waste from industry mining activity and residues from pesticides	Kansas R. at Lawrence	0.003	1970
			Kansas R. at Topeka	0.008	1970
			Kansas R. and Lawrence R.	0.002-0.010	1970
			Kansas R.	0.002-0.008	
			Northeast Basin	Mean Positive: 0.034	1962-67
			North Atlantic Basin	0.047	
			Southeast Basin	0.035	
			Tennessee R. Basin	0.050	
			Ohio R. Basin	0.066	
			Lake Erie Basin	0.308	
			Upper Mississippi R. Basin	0.069	
			Western Great Lakes Basin	0.037	
			Missouri R. Basin	0.123	
			Lower Mississippi R. Basin	0.091	
			Colorado R. Basin	0.053	
			Western Gulf Basin	0.022	
			Pacific Northwest Basin	0.068	
			Great Basin	0.020	
			Alaska Basin	0.034	
Barium	Weathering of rock	Brines from oil well waste and effluents from mining areas.	Northeast Basin	Mean Positive: 0.021	1962-67
			North Atlantic Basin	0.025	
			Southeast Basin	0.026	
			Tennessee R. Basin	0.025	
			Ohio R. Basin	0.043	
			Lake Erie Basin	0.042	
			Upper Mississippi R. Basin	0.039	
			Western Great Lakes Basin	0.015	
			Missouri R. Basin	0.063	
			Lower Mississippi R. Basin	0.090	
			Colorado R. Basin	0.060	
			Western Gulf Basin	0.067	
			Pacific Northwest Basin	0.027	
			California Basin	0.042	
			Great Basin	0.041	
			Alaska Basin	0.017	
			Coosa R. Below Rome, Ga.	0.340 (max. conc.)	

	Sources		Concentrations in U.S. Waters			
Agent	**Natural**	**Man-Made**	**Basin Areas**		**Concentrations (mg/l)**	
Beryllium	Weathering of mineral beryl	Effluent from atomic reactors, metallurgy, aircraft, rocket and missile fuel industries	Northeast Basin	Mean Positive:	0.002	1962-67
			North Atlantic Basin		0.00012	
			Southeast Basin		0.00005	
			Tennessee R. Basin		0.00016	
			Ohio R. Basin		0.00028	
			Lake Erie Basin		0.00017	
			Western Great Lakes Basin		0.00005	
			Missouri R. Basin		0.00023	
			Pacific Northwest Basin		0.00002	
			Monongahela R. at Pittsburgh, Pa.		0.00122 (max. conc.)	1962-1967
Bismuth	Weathering of rock	Metallurgy; medicinal	Principal rivers of U.S.	None detected		1958-59
			Streams in California		0.0006-0.0008	
Boron	Weathering of boron-bearing rock	Industrial effluents of weather proofing wood, tire proofing fabrics, manufacturing glass and porcelain, production of leather and carpets, cosmetics, photography, artificial gems, high energy fuels, bactericides and fungicides	Northeast Basin	Mean Positive:	0.032	1962-67
			North Atlantic Basin		0.042	
			Southeast Basin		0.029	
			Tennessee R. Basin		0.024	
			Ohio R. Basin		0.067	
			Lake Erie Basin		0.210	
			Upper Mississippi R. Basin		0.105	
			Western Great Lakes Basin		0.019	
			Missouri R. Basin		0.154	
			Lower Mississippi R. Basin		0.131	
			Colorado R. Basin		0.179	
			Western Gulf Basin		0.289	
			Pacific Northwest Basin		0.030	
			California Basin		0.143	
			Great Basin		0.084	
			Alaska Basin		0.028	
			Colorado R. at Yuma, Ariz.		1.800 (max. conc.)	

	Sources		Concentrations in U.S. Waters		
Agent	Natural	Man-Made	Basin Areas	Concentrations (mg/l)	
Bromine		Rainfall, combustion of leaded gasoline containing ethylene dibromide, effluents from industries producing salt, chemical medicinal compounds, and disinfectants	Lake Superior, Mich.	0.007-0.033	1969
			Lake Superior tributaries	0.005-0.260	
			Lake Michigan	0.011-0.021	
			Lake Huron	0.013-0.029	
			St. Clair R.	0.019-0.039	
			Lake St. Clair	0.045-0.055	
			Detroit R.	0.020-0.028	
			Lake Erie	0.020-0.054	
			Lake Ontario	0.038-0.077	
Cadmium	Weathering of rock	Effluents from industries using cadmium such as metallurgy, electroplating, ceramics, pigmentation, photography, nuclear reactors, insecticides and antihelminthics	Northeast Basin	Mean Positive: 0.005	1962-67
			North Atlantic Basin	0.003	
			Southeast Basin	0.005	
			Ohio R. Basin	0.007	
			Lake Erie Basin	0.050	
			Upper Mississippi R. Basin	0.006	
			Western Great Lakes Basin	0.005	
			Colorado R. Basin	0.002	
			Western Gulf Basin	0.010	
			Pacific Northwest Basin	0.005	
			Great Basin	0.001	
			Cuyahoga R. at Cleveland, Ohio	0.120 (max. conc.)	

	Sources		Concentrations in U.S. Waters		
Agent	Natural	Man-Made	Basin Areas	Concentrations (mg/l)	
Chloride	Weathering of rock, animal sewage, sea-water contamination of groundwater supplies	Enters natural waters from salts spread on fields for agricultural purposes, and industrial effluents such as paper mills, water softening plants, galvanizing plants, oil wells, petroleum refineries, and highway deicing	New Jersey surface waters 1) spring flow 2) summer flow	 8.5-20.0 6.7-15.5	 1968
			New Jersey surface water Big Flat Brook: Calcareous - Site 1 Acidic - Site 2	 11.5 4.0	
			Santa Anna R. at Prado Dam	89-152	1952-68
			Platte R. near Venice, Neb. Platte R. near Ashland, Neb. Elkhorn R. at Q St., Neb.	6.8 6.8 10.1	
			Surface waters of 98 U.S. rivers	1-702	1961
			Surface waters of U.S. rivers	5.5 8.0 10.0 83.0	Aug. 1961 Sept. 1961 Oct. 1961 1961
			Drinking water for 163 metropolitan areas of U.S.	20.1	1950-51
			Seven Maine rivers (highway deicing)	July 0.5-7.1 Oct. 0.5-7.8 Apr. 0.5-3.8	1965-67
			Lower Detroit R	7-70	
			Colorado R. Basin (max. at Dolores R. near Cisco, Utah)	1.0-1840.0	1964-65
			Pacific Slope Basins in Calif. (max. at Salt Slough near Los Banos, Calif.)	0.0-524	

	Sources		Concentrations in U.S. Waters			
Agent	Natural	Man-Made	Basin Areas	Concentrations (mg/l)		
Chromium		Industrial effluents using chromium such as metal pickling and plating operations, anodizing aluminum, leather processing, manufacture of paints, dyes, explosives, ceramics, paper, glass, corrosion inhibitors and many others	Tennessee R. Basin	Mean Positive:	0.006	1962-67
			Ohio R. Basin		0.007	
			Lake Erie Basin		0.012	
			Upper Mississippi R. Basin		0.007	
			Western Great Lakes Basin		0.006	
			Missouri R. Basin		0.017	
			Lower Mississippi R. Basin		0.016	
			Colorado R. Basin		0.016	
			Western Gulf Basin		0.025	
			Pacific Northwest Basin		0.006	
			California Basin		0.015	
			Great Basin		0.004	
			Alaska Basin		0.009	
			St. Lawrence R. at Massena, N.Y.		0.112 (max. conc.)	1962-67
Copper	Weathering of rock	Corrosive action of water in copper pipes, industrial effluents such as discharged from textile mills, pigmentation, tanning, photography, engraving, electroplating, insecticides, fungicides and in many other industrial processes	Northeast Basin	Mean Positive:	0.015	1962-67
			North Atlantic Basin		0.017	
			Southeast Basin		0.014	
			Tennessee R. Basin		0.011	
			Ohio R. Basin		0.023	
			Lake Erie Basin		0.011	
			Upper Mississippi R. Basin		0.014	
			Western Great Lakes Basin		0.007	
			Missouri R. Basin		0.017	
			Lower Mississippi R. Basin		0.019	
			Colorado R. Basin		0.010	
			Western Gulf Basin		0.011	
			Pacific Northwest Basin		0.009	
			California Basin		0.012	
			Great Basin		0.012	
			Alaska Basin		0.009	
Cobalt	Weathering of rock	Industrial effluents from plants using cobalt such as metallurgical firms, ceramics and glass manufacturing, the tungsten carbide tool industry and others	Northeast Basin	Mean Positive:	0.014	1962-67
			North Atlantic Basin		0.009	
			Southeast Basin		0.001	
			Ohio R. Basin		0.019	
			Lake Erie Basin		0.033	
			Upper Mississippi R. Basin		0.018	
			Western Great Lakes Basin		0.011	
			Missouri R. Basin		0.008	
			Lower Mississippi R. Basin		0.036	
			Colorado R. Basin		0.011	
			Pacific Northwest Basin		0.008	
			Allegheny R. at Pittsburgh, Pa.		0.048 (max. conc.)	

	Sources		Concentrations in U.S. Waters		
Agent	Natural	Man-Made	Basin Areas	Concentrations (mg/l)	
Fluoride	Weathering of rock containing fluoride	Industrial effluents from plants using fluorides in the production of steel, disinfectants, preserved wood and mucilages, glass and enamels, chemicals It is added to many public water supplies to reduce incidence of cavities in teeth.	Surface waters of U.S.	0.1	June 1961
				0	Sept 1961
				0.4	Oct. 1961
				0.4	1961
			Drinking water of 163 metropolitan areas in U.S.	Average: 0.25	1950-51
			Rocky Creek and East Gallatin R. (various locations along rivers from sources of pollution)	3.6-16.0	
			Colorado R. Basin (max at Virgin R. below Littlefield, Ariz.)	0.0-0.9	1964-65
			Pacific Slope Basins in Calif. (max. at Colusa Trough near Colusa, Calif.)	0.1-0.7	
			Great Basin excluding Great Salt Lake (max. at Humboldt R. near Rye Patch, Nev.)	0.0-0.8	
			Pacific Slope Basins in Washington and Upper Columbia R. Basin (max. at Crab Creek near Smyrna, Washington)	0.1-0.7	
			Pacific Slope Basins in Ore. and Lower Columbia R. Basin (max at Walla Walla R. near Touchet, Wash.)	0.0-0.5	
			Snake R. Basin (max. at Snake R. at King Hill, Idaho)	0.0-0.9	
			Alaska (max. at Tonsina R. at Tonsina, Alaska)	0.0-0.6	
			North Atlantic Slope Basins (max. at Wissachickon Creek at Fort Washington, Pa.)	0.0-1.4	1964-65
			South Atlantic Slope Basins (max. at Alafia R. at Lithia, Fla.)	0.0-17-0	
			Ohio R. Basin (max. at Tuscarawas R. at Newcomerstown, Ohio)	0.0-4.2	1964-65
			St. Lawrence R. Basin (max at Black R. at Elyria, Ohio)	0.0-1.7	

Agent	Sources		Concentrations in U.S. Waters		
	Natural	Man-Made	Basin Areas	Concentrations (mg/l)	
			Missouri R. Basin (max at Cedar Creek near Columbia, Mo.	0.0-3.4	1964-65
			Hudson R. and Upper Mississippi R. Basins (max at Souris R. near Verendrye, N. Dak.)	0.0-1.2	
			Lower Mississippi R. Basin (max at Center Creek at Oronogo, Mo.)	0.0-15.0	
			Western Gulf of Mexico Basins (max at Double Mountain Fork Brazos R. at Justiceburg, Tex.)	0.0-2.1	
			Illinois surface waters (max at Illinois R. at Peoria-1964)	0.0-2.4	1956-66
Iron	Leaching of iron salts from soil and rock into both ground and surface waters	Acid mine drainage, the effluents from the many industries using iron in their processing, and the oxidation of iron used in such items as ships, automobiles, refrigerators and many others	Northeast Basin	Mean Positive: 0.051	1962-67
			North Atlantic Basin	0.019	
			Southeast Basin	0.120	
			Tennessee R. Basin	0.037	
			Ohio R. Basin	0.028	
			Lake Erie Basin	0.035	
			Upper Mississippi R. Basin	0.035	
			Western Great Lakes Basin	0.022	
			Missouri R. Basin	0.037	
			Lower Mississippi R. Basin	0.069	
			Colorado R. Basin	0.040	
			Western Gulf Basin	0.173	
			Pacific Northwest Basin	0.032	
			California Basin	0.046	
			Great Basin	0.070	
			Alaska Basin	0.025	
			Sabine R. near Ruliff, Tex.	0.952 (max. conc.)	

	Sources		Concentrations in U.S. Waters		
Agent	Natural	Man-Made	Basin Areas	Concentrations (mg/l)	
Lead	Weathering of limestone and galena	Corrosion of lead in pipes and near industrial outfalls from industrial plants using lead in their processes	Northeast Basin	Mean Positive: 0.017	1962-67
			North Atlantic Basin	0.014	
			Southeast Basin	0.008	
			Tennessee R. Basin	0.017	
			Ohio R. Basin	0.030	
			Lake Erie Basin	0.039	
			Upper Mississippi R. Basin	0.033	
			Western Great Lakes Basin	0.014	
			Missouri R. Basin	0.039	
			Lower Mississippi R. Basin	0.037	
			Colorado R. Basin	0.032	
			Western Gulf Basin	0.004	
			Pacific Northwest	0.015	
			California Basin	0.004	
			Great Basin	0.018	
			Alaska Basin	0.012	
			Ohio R. at Evansville, Ind.	0.140 (max. conc.)	
Lithium	Weathering of rock containing lithium	Effluents from industries using lithium in the manufacture of metal alloys, medicines, some types of glass and lithium hydroxide for storage batteries	Apalachicola R. Near Blounstown, Fla.	0.000075-0.00070	1958-5
			Atchafalaya R. at Krotz Springs, La.	0.0019-0.0045	
			Colorado R. at Yuma, Ariz.	0.035-0.037	
			Columbia R. above Dalles, Ore.	0.00059-0.0039	
			Hudson R. at Green Island, N.Y.	0.00028-0.0022	
			Mississippi R. near Baton Rouge, La.	0.0018-0.0049	
			Mobile R. at Mt. Vernon Landing, Ala.	0.00014-0.0024	
			Sacramento R. at Sacramento, Cal.	0.00066-0.0021	
			Susquehanna R. at Conowingo, Md.	0.0034-0.0035	
			Yukon R. at Mountain Village, Alaska	0.0020	
			North Atlantic Slope Basins (max. at Raritan R. at Manville, N. J.)	0.0-0.027	1964-65

Agent	Sources		Concentrations in U.S. Waters		
	Natural	Man-Made	Basin Areas	Concentrations (mg/l)	
Nitrate	Animal fecal material	Effluents from plants producing chemical fertilizer. Application of chemical fertilizers on agricultural lands that are then leached into the surface and percolating ground waters. Application of fertilizers on residential lawns and gardens. Municipal treatment plant effluent	Surface waters of 98 U.S. rivers	Range: 0.1-10.1	1960-
			Surface waters of U.S.	4.7	Aug. 19
				0.5	Sept 19
				3.2	Oct 19
				1.4	19
			Missouri R. water		1951-
			in Omaha, Neb.	0.2-8.6 monthly	
			in Kansas City, Mo.	1.8 "	
			in St. Louis County, Mo.	1.0-9.0 "	
			in St. Louis, Mo.	2.1-11.3 "	
			Hudson R.	0.2-1.0	19
			Iowa	10.0-100.0	19
			Ohio, 10% of municipal water supply	1.0	July 19
			Mahoning R.	10	March 19
			Ohio rural water supplies	10.0-100.0	19
			Drinking water in 163 metropolitan areas in U.S.	Average: 2.3	1950-
			Missouri wells	5-300	
			Lake Erie	1.0	1
			Colorado R. basin (max. at Gila R. below Gillespie Dam, Ariz.)	0.00-60.0	1
			Pacific Slope Basins in Calif. (max. at Salinas R. near Sprechles, Cal.)	0.0-38.0	
			Great Basin excluding Great Salt Lake (max. at Jordan R. at Salt Lake City, Utah)	0.0-17.0	
			Pacific Slope Basins in Washington and Upper Columbia R. Basin (max. at Flett Creek at Tacoma, Wash.)	0.0-12.0	1
			Pacific Slope Basins in Oregon and Lower Columbia R. Basin (max. at Walla Walla R. near Touchet, Wash.)	0.0-4.2	

Agent	Sources		Concentrations in U.S. Waters		
	Natural	Man-Made	Basin Areas	Concentrations (mg/l)	
			Snake R. Basin (max. at Palouse R. at Hooper, Wash.)	0.0-14.0	
			Alaska (max. at Trail R. near Lowing, Alaska	0.0-2.8	
			North Atlantic Slope Basins (max. at Rockaway R. at Pine Brook, N.J.)	0.0-62.0	
			South Atlantic Slope and Eastern Gulf of Mexico Basins (max. at Plantation Rd. Canal near Fort Lauderdale, Fla.)	0.0-620.0	
			Ohio R. Basin (max. at Great Miami R. at Elizabethtown, Ohio)	0.0-23.0	1964-
			St. Lawrence R. Basin (max. at Black R. at Elyria, Ohio)	0.2-113.0	
			Missouri R. Basin (max. at Horse Creek near Vale, S. Dak.)	0.0-77.0	1964-
			Hudson R. and Upper Mississippi R. Basins (max. at Blue Earth R. near Rapidan, Minn.)	0.0-302.0	
			Lower Mississippi R. Basin (max. at Fountain Creek at Pueblo, Colo.)	0.0-302.0	
			Western Gulf of Mexico Basins (max. at Yegua Creek near Somerville, Tex.)	0.0-54.0	
			Illinois surface waters (max. at Illinois R. Meredosia-1962)	0.0-48.4	1956-6
			Lehigh R. Basin (max. at Black Creek near Weatherly-1963)	0.0-23.0	1945-6

	Sources		Concentrations in U.S. Waters		
Agent	Natural	Man-Made	Basin Areas	Concentrations (mg/l)	
Nickel			New Jersey surface waters		1968
			1) spring flow	0.002-0.009	
			2) summer flow	0.001-0.017	
			New Jersey surface waters Big Flat Brook		
			Calcareous - Site 1	0.006	
			Acidic - Site 2	0.001	
			Lower Mississippi R. Basin: Californiaa Gulch and Arkansas R. at Malta, Colo. (max. at California Gulch)	0.0-0.069	1964-65
Phosphorus	The decomposition of organic matter	Siltation. Applied fertilizer. Effluents of plants making fertilizers, matches armaments, organic chemicals, ortho-phosphoric acid, food supplement in animal foods, water softeners, and in metalurgy	Northeast Basin	Mean Positive: 0.044	1962-67
			North Atlantic Basin	0.048	
			Southeast Basin	0.043	
			Tennessee R. Basin	0.042	
			Ohio R. Basin	0.130	
			Lake Erie Basin	0.153	
			Upper Mississippi R. Basin	0.243	
			Western Great Lakes Basin	0.031	
			Missour R. Basin	0.353	
			Lower Mississippi R. Basin	0.081	
			Colorado R. Basin	0.121	
			Western Gulf Basin	0.173	
			Pacific Northwest Basin	0.047	
			California	0.083	
			Great Basin	0.037	
			Alaska Basin	0.040	

	Sources		Concentrations in U.S. Waters	
Agent	Natural	Man-Made	Basin Areas	Concentrations (mg/l)
Manganese	Leached from rock into groundwaters	Mining effluents from from industries using manganese in the production of steel alloys, dry cell batteries, glass and ceramics, paints and varnish, inks, matches, fireworks and in agriculture to nourish manganese deficient soils	Northeast Basin	Mean Positive: 0.0035 1962-67
			North Atlantic Basin	0.0027
			Southeast Basin	0.0028
			Tennessee R. Basin	0.0037
			Ohio R. Basin	0.232
			Lake Erie Basin	0.138
			Upper Mississippi R. Basin	0.0098
			Western Great Lakes Basin	0.0023
			Missouri R. Basin	0.0138
			Lower Mississippi R. Basin	0.009
			Colorado R. Basin	0.012
			Western Gulf Basin	0.010
			Pacific Northwest Basin	0.0028
			California Basin	0.0028
			Great Basin	0.078
			Alaska	0.0018
Mercury	Leaching of rock and volatilization	Effluents to air emissions from chlor-alkali plants, applications of pesticides, fuel burning, catalytic processes, ore refining, sewage treatment, waste incineration, phosphate rock, processing, paint manufacture, and use and breakage of mercury-containing devices, and many others	Range of Hg found in all of the States in Oct & Nov, 1970	0.5 ug/l-6.9 ug/l
Molybdenum	Mineral weathering	Mining and processing molybdenum ore. Effluents of industries using molybdenum in their operation such as metallurgical, glass, ceramics, pigment production, fertilizers and other	Northeast Basin	Mean Positive: 0.025 1962-67
			North Atlantic Basin	0.033
			Southeast Basin	0.015
			Tennessee R. Basin	0.025
			Ohio R. Basin	0.070
			Lake Erie Basin	0.068
			Upper Mississippi R. Basin	0.088
			Western Great Lakes Basin	0.028
			Missouri R. Basin	0.083
			Lower Mississippi R. Basin	0.095
			Colorado R. Basin	0.130
			Western Gulf Basin	0.024
			Pacific Northwest Basin	0.030
			California Basin	0.045
			Great Basin	0.145
			Alaska Basin	0.017

	Sources		Concentrations in U.S. Waters	
Agent	Natural	Man-Made	Basin Areas	Concentrations (mg/l)
Selenium	-----	Traces in effluents from plants using silver to produce such items as pigmentation for paints, dyes, glass, components in rectifiers, semi-conductors	Selected drinking water supplies in:	
			Connecticut	0.010
			Maine	0.010
			New Hampshire	0.010
			Vermont	0.010
			Delaware	0.010
			New York	0.010
			Pennsylvania	0.010
			Kentucky	0.010
			Maryland	0.010
			North Carolina	0.010
			Virginia	0.010
			W. Virginia	0.010
			Alabama	0.010
			Florida	0.010
			Georgia	0.010
Silver	Weathering of rocks	Trace amounts in effluents from industrial plants using silver such as electroplating plants, food and beverage plants, photographic chemicals producers, ink manufacturing, antiseptics for medicinal purposes	Northeast Basin	Mean Positive: 0.0019 1962-67
			North Atlantic Basin	0.0009
			Southeast Basin	0.0004
			Ohio R. Basin	0.0021
			Lake Erie Basin	0.0053
			Upper Mississippi R. Basin	0.0034
			Western Great Lakes Basin	0.0014
			Missouri R. Basin	0.0012
			Lower Mississippi R. Basin	0.0043
			Colorado R. Basin	0.0058
			Pacific Northwest Basin	0.0009
			Great Basin	0.0003
			Alaska Basin	0.0011

	Sources		Concentrations in U.S. Waters		
Agent	Natural	Man-Made	Basin Areas	Concentrations (mg/l)	
Uranium	Weathering of rock	Mining and processing of uranium in the manufacture of atomic weapons, the production of radioisotopes in piles and reactors, use of radioisotopes in medical therapy, scientific research photography, and in many chemical processes	Walker R. Calif.	0.0009	
			Weber R., Utah	0.0013	
			Little Cottonwood Creek, Utah	0.002	
			Williams Creek, Idaho	0.0003	
			Blye Mountain, Calif.	0.00004	
			Well-Arches National Monument, Utah	0.0050	
			Hot Spring-Pyramid Lake, Nevada	0.00008	
			Truchee R., Nevada	0.0046	
			Walker R., Nevada	0.016	
			Humbolt R., Nevada	0.0072	
			Carson R., Nevada	0.0047	
			Sevier R., Utah	0.0081	
			Lake Tahoe, Calif.	0.0008	
			Pyramid Lake, Nevada (South end)	0.016	
			Pyramid Lake, Nevada (North end)	0.025	
			Walker Lake	0.077	
			Mono Lake	0.139	
			Principal rivers of U.S.	none detected	1958-59
			North Atlantic Slope Basins (max at Susquehanna R. at Harrisburg, Pa. and Potomac R. at Hancock, Md.)	0.0004-0.0006	1960-61
			St. Lawrence R. Basin Genessee R. at Rochester, N.Y.	0.0004	1960-61
			South Atlantic Slope Basins (max at Yadkin R. at Yadkin College, N.C. and St. Johns R. at Christmas, Fla)	0.0004-0.0007	1960-61
			Gulf of Mexico Basin (max at Missouri R. at Nebraska City, Neb.)	0.0004-0.0006	1960-61
			Colorado R. at Yuma, Ariz.	0.0076	
			Pacific Coast Basins (max at San Joaquin R. at Vernalis, Calif.)	0.0004-0.008	
			Yukon R. at Rampart, Alaska	0.0014	
			Red R. (N) at Grand Forks, N.D.	0.0014	

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Agent	Sources		Concentrations in U.S. Waters			
	Natural	Man-Made	Basin Areas	Concentrations (mg/l)		
Vanadium	Weathering of rock	Leaching of soils. Degradation of plants and animals. Effluents from plants making steel, glass, mordant used in dyeing and printing fabrics, and other products	Northeast Basin	Mean Positive:	0.009	1962-67
			North Atlantic Basin		0.012	
			Southeast Basin		0.010	
			Tennessee R. Basin		0.022	
			Lake Erie Basin		0.054	
			Upper Mississippi R. Basin		0.020	
			Missouri R. Basin		0.171	
			Lower Mississippi R. Basin		0.025	
			Colorado R. Basin		0.105	
			Western Gulf Basin		0.009	
			Pacific Northwest Basin		0.013	
			California Basin		0.030	
			Alaska Basin		0.032	
Zinc	Weathering of rock	Mining and processing of ore. Effluents from plants making pharmaceuticals, dyes, insecticides, and many other products	Northeast Basin	Mean Positive:	0.096	1962-67
			North Atlantic Basin		0.049	
			Southeast Basin		0.052	
			Tennessee R. Basin		0.028	
			Ohio R. Basin		0.081	
			Lake Erie		0.205	
			Upper Mississippi R. Basin		0.045	
			Western Great Lakes Basin		0.024	
			Missouri R. Basin		0.039	
			Lower Mississippi R. Basin		0.085	
			Colorado R. Basin		0.051	
			Western Gulf Basin		0.092	
			Pacific Northwest Basin		0.040	
			California Basin		0.016	
			Great Basin		0.044	
			Alaska Basin		0.028	

IV. Measurement and Classification of Waters 304(a)(2)(C)

A. Measurement Techniques

Methods included within the following section cover a variety of measurements and techniques. For some pollutants, several methods are cited for measurement or detection. Each method is summarized and presents a synopsis and is not intended to give a detailed presentation. Rather an outline of the technique is followed immediately by the pertinent references for an extensive review of the procedure.

The techniques are divided into three areas; physical-chemical, biological, and bioassays. It is not within the scope of this document to detail precisely or to recommend a specific method. Often circumstances such as sources and mixtures of pollutants, cost of test, and skill of the personnel performing the test will dictate the preferred procedure.

ACIDITY

Principal Detection Technique: Titration

Summary of Method: Sample is titrated to a final pH of 8.3. Results reported as mg of $CaCO_3$ per liter.

Limitations:

Interferences: Not applicable to acid samples from mine drainage

Statistical Characteristics: (By 40 analysts in 17 laboratories) **Over Observed Range of:**

Accuracy:	**Bias**	**Acidity, as mg/l $CaCO_3$**
	+2.77%	20
	+0.52%	21
Precision:	**Standard Dev.**	
	1.79 mg/l $CaCO_3$	20
	1.73	21

Time of Measurement (Maximum frequency, recovery period, etc.): Not stated, but fast.

Data Outputs: Analog signal displayed on meter.

Special Sampling Requirements (Collection, Storage, Handling): Refrigerate sample at 4°C.
Maximum holding time is 24 hours

References:

1. Methods for Chemical Analysis of Water and Wastes. 1971. EPA National Environmental Research Center, Cincinnati, Ohio.

2. Annual Book of ASTM Standards, part 23 (Method designation D1067). 1970. Society for Testing and Materials. Philadelphia, Pennsylvania.

ALKALINITY

Name of Measurement Method: Electrometric Titration

Principal Detection Technique: Electrometric

Purpose of Measurement (Important Applications): Drinking waters, ambient surface waters, domestic and industrial wastes, and saline waters.

Summary of Method: Unaltered sample is titrated to an electrometrically determined end point of pH 4.5. Sample must not be filtered, diluted, concentrated, or otherwise altered.

Limitations:

Range of Applicability: All concentrations

Interferences: Salts of weak acids, oils and greases

Pitfalls; Special Precautions: Analyze sample as soon as possible after collection, preferably within a few hours.

Statistical Characteristics: Over Observed Range of:

Accuracy:	Bias	Alkalinity in mg/l $CaCO_3$
	+22.29%	9
	- 8.19%	113
Precision:	Std. Dev., mg/l $CaCO_3$	
	1.4	9
	5.28	113

Time of Measurement (Maximum frequency, recovery period, etc.):: Not stated, but rapid.

Data Outputs: Meter (analog voltage)

Special Sampling Requirements (Collection, Storage, Handling): Refrigerate at 4°C. Maximum holding time 24 hours.

References:

1. Methods for Chemical Analysis of Water and Wastes. 1971. EPA National Environmental Research Center, Cincinnati, Ohio.

2. Annual Book of ASTM Standards, part 23 (Method designation D1067). 1970. Society for Testing and Materials. Philadelphia, Pennsylvania.

Total Alkalinity

Name of Measurement Method: Methyl Orange

Principal Detection Technique: Titration with colorimetric detection of end point.

Purpose of Measurement (Important Applications): Surface waters, saline waters.

Summary of Method: A Technicon Autoanalyzer is used, employing methyl orange as indicator. This is dissolved in a weak buffer at pH of 3.1, just below the equivalence point. Addition of alkalinity causes loss of color proportional. Color measurement made by colorimeter at approximately 550 nm.

Limitations:

Range of Applicability: 10 to 200 mg/l expressed as $CaCO_3$

Statistical Characteristics:

Precision: Std. dev. was ±0.5 mg/l $CaCO_3$ (in one lab) using concentrations of 15, 57, 154, and 193 mg/l $CaCO_3$

Time of Measurement (Maximum frequency, recovery period, etc.): 30 min. warm-up, about 1 min. per determintation

Calibration Requirements: Requires preparation of standard curve of peak heights vs. concentration

Data Outputs: Strip recorder (analog voltage)
Special Sampling Requirements (Collection, Storage, Handling): Refrigerated at 4°C; sample analyzed as soon as possible.

References:

1. Methods for Chemical Analysis of Water and Wastes. 1971. EPA National Environmental Research Center, Cincinnati, Ohio.

2. Technicon Autoanalyzer Methodology. 1961. Bulletin 1261.

ARSENIC

Name of Measurement Method: Diethyldithiocarbamate method

Principal Detection Technique: Colorimetry

Purpose of Measurement (Important Applications): Most fresh water

Summary of Method: Arsenic in sample is reduced to arsine, AsH_3, in acid solution. The arsine is scrubbed to remove sulfide, and is absorbed in a solution of diethyldithiocarbamate dissolved in pyridine. The red complex thus formed is measured in a spectrophotometer at 535 nm.

Limitations:

Range of Applicability: At or above 10 ug/l As. (If arsenic is organically bound, consult Standard Methods, 13th Edition).

Interferences: High concentrations of chromium, cobalt, copper, mercury, molybdenum, nickel, or silver.

Pitfalls; Special Precautions: Difficulties may be encountered with certain industrial wastes containing volatile substances. High sulfur content of wastes may exceed removal capacity of lead acetate scrubber.

Statistical Characteristics: In 46 laboratories:

		Over Observed Range Of:
Accuracy:	Relative error = 0%	40 ug/l
Precision:	Relative Std. Dev. = 13.8	40 ug/l as As

Data Outputs: Chart or meter (analog voltage)

References:

1. Methods for Chemical Analysis of Water and Wastes. 1971. EPA National Environmental Research Center, Cincinnati, Ohio.

2. Standard Methods for Examination of Water and Wastewater. 1971. 13th Edition. American Public Health Association, Washington, D. C.

BIOCHEMICAL OXYGEN DEMAND (BOD)

Name of Measurement Method: Modified Winkler with Full Bottle Technique.

Principal Detection Technique: Titration

Purpose of Measurement (Important Applications): Ambient surface water, domestic and industrial waste waters (especially sewage treatment plant effluent).

Summary of Method: Sample of waste, diluted as appropriate, is incubated for 5 days in darkness at 20°C. The reduction in dissolved oxygen concentration during this period yields a measure of the biochemical oxygen demand.

Limitations: See summary for Dissolved Oxygen (Modified Winkler Method).

Statistical Characteristics:

Precision:	Over Observed Range Of:
Seventy-seven analysts in fifty-three labs analyzed samples of natural water plus an exact increment of biodegradable compounds. At a mean of 194 mg/l BOD, the standard deviation was ±40 mg/l	An unspecified range with mean value of 194 mg/l BOD

Time of Measurement (Maximum Frequency, Recovery Period, etc.):

Five to six days per determination.

Comments by Users: Because of local conditions, types of samples to be tested, and variabilities of the bioassay procedures, no specific standard test for BOD has been selected by EPA.

References:

1. Methods for Chemical Analysis of Water and Wastes. 1971. EPA National Environmental Research Center, Cincinnati, Ohio.

2. Standard Methods for Examination of Water and Wastewater. 1971. 13th Edition. American Public Health Association, Washington, D. C.

BIOCHEMICAL OXYGEN DEMAND (BOD)

Name of Measurement Method: DO Probe

Principal Detection Technique: Electrometric method.

Purpose of Measurement (Important Applications): See summary of BOD determination using Modified Winkler Method.

Summary of Method: See BOD determination using Modified Winkler Method.

Limitations: (See Summary for Dissolved Oxygen (Probe Method)).

Statistical Characteristics: See DO determination using Modified Winkler Method.

Comments by Users: Because of local conditions, types of samples to be tested, and variabilities of the bioassay procedures, no specific standard test for BOD has been selected by EPA.

Data Outputs: Electrical signal displayed on meter.

Special Sampling Requirements (Collection, Storage, Handling): Refrigerate at 4°C. Maximum storage time - 6 hours.

References:

1. Methods for Chemical Analysis of Water and Wastes. 1971. EPA National Environmental Research Center, Cincinnati, Ohio.
2. Standard Methods for Examination of Water and Wastewater. 1971. 13th Edition. American Public Health Association, Washington, D. C.

ORGANIC CARBON (TOTAL AND DISSOLVED)

Principal Detection Technique: Infrared spectroscopy

Purpose of Measurement (Important Applications): Ambient surface waters, domestic and industrial wastes, saline waters; used for assessing potential oxygen-demanding load of organic matter.

Summary of Method: A micro sample of the wastewater to be analyzed is injected into a catalytic combustion tube which is enclosed by an electric furnace thermostated at 950 C. The water is vaporized and the carbonaceous material is oxidized to Carbon dioxide (CO_2) and steam in a carrier stream of pure oxygen or air. The oxygen flow carries the steam and CO_2 out of the furnace where the steam is condensed and the condensate removed. The CO_2, oxygen, and remaining water vapor enter an infrared analyzer sensitized to provide a measure of CO_2. The amount of CO_2 present is directly proportional to the concentration of carbonaceous material in the injected sample.

Limitations:

Range of Applicability: 1 to 140 mg/l total carbon.

Interferences: Carbonates, bicarbonates

Pitfalls; Special Precautions: Since sample is injected into apparatus by syringe, the needle opening limits the size of particles reaching combustor/detector.

Statistical Characteristics:

Twenty-eight analysts in twenty-one laboratories analyzed distilled water solutions containing exact concentrations of oxidizable organic compounds, with the following results:

Known Carbon Conc. as TOC, mg/liter	Precision as Standard Deviation TOC, mg/liter	Accuracy as Bias, %	Bias, mg/liter
4.9	3.93	+15.27	+ .75
107	8.32	+ 1.01	+1.08

Time of Measurement: Not stated; assumed to be rapid (several samples per hour).

Special Sampling Requirements (Collection, Storage, Handling): None indicated. Maximum holding time - 7 days.

References::

1. Methods for Chemical Analysis of Water and Wastes. 1971. EPA National Environmental Research Center, Cincinnati, Ohio.

2. Standard Methods for Examination of Water and Wastewater. 1971. 13th Edition. American Public Health Association, Washington, D. C.

3. ASTM Standards. 1970. Part 23, Water, **Atmospheric** Analysis.

CHLORIDE

Principal Detection Technique: Titration

Purpose of Measurement (Important Applications): Drinking waters, ambient surface waters. Domestic and industrial wastes, saline waters.

Summary of Method: Dilute mercuric nitrate solution is added to an acidified sample in the presence of mixed diphenylcarbazone - bromophenol blue indicator. The end point of the titration is the formation of the blue-violet diphenylcarbazone complex.

Limitations:

Range of Applicability: All concentration ranges of chloride

Interferences: Sulfites. If presence is suspected, oxidize with hydrogen peroxide.

Statistical Characteristics: By 42 analysts in 18 laboratories

	Over Observed Range Of:
Accuracy: Percent bias ranged from 13.50 (at 10 mg/l) to 1.10 (at 398 mg/l).	17, 18, 91, 97, 392, 398 mg/l
Precision: Standard Deviations ranged from 1.32 mg/l (at 18 mg/l Cl^-) to 11.8 mg/l (at 398 mg/l Cl^-)	Same

Data Outputs: Visual observation and manual recording (or may use automated titration, yielding an analog voltage recorded on chart).

Special Sampling Requirements (Collection, Storage, Handling): None indicated. Maximum holding time - 7 days.

References:

1. Methods for Chemical Analysis of Water and Wastes. 1971. EPA National Environmental Research Center, Cincinnati, Ohio.

2. Standard Methods for Examination of Water and Wastewater. 1971. 13th Edition, American Public Health Association, Washington, D. C.

3. ASTM Standards. 1970. Part 23, Water, Atmospheric Analysis.

CHLORIDE

Name of Measurement Method: Automated Ferricyanide Method

Principal Detection Technique: Titration, using colorimeter

Purpose of Measurement (Important Applications): Ambient surface waters, domestic and industrial wastes, saline waters.

Summary of Method: Thiocyanate ion (SCN) is liberated from mercuric thiocyanate through sequestration of mercury by chloride ion to form un-ionized mercuric chloride ion. In the presence of ferric ion the thiocyanate SCN forms highly colored ferric thiocyanate in concentration proportional to the original chloride concentration.

The determination may be performed automatically using a Technicon AutoAnalyzer employing a colorimeter with 380 nm filter for endpoint detection.

Limitations:

Range of Applicability: 1 to 250 mg/l chloride

Statistical Characteristics:	Over Observed Range Of:
In a single laboratory:	
Precision: Standard deviation ±0.3 mg/l chloride	1; 100; and 250 mg/l chloride
Time of Measurement: About 2 minutes per sample, with 30 min. warm-up.	

Data Outputs: Analog voltage, recorded on strip chart

Special Sampling Requirements (Collection, Storage, Handling): None. Maximum holding time - 7 days.

References:

1. Methods for Chemical Analysis of Water and Wastes. 1971. EPA National Environmental Research Center, Cincinnati, Ohio.

2. O'Brien, J.E. 1962. Automatic Analysis of Chlorides in Sewage. Waste Engineering, 33: 670-672.

CHLORINE REQUIREMENT

Principal Detection Technique: Colorimetry or Electrometric Determination

Purpose of Measurement (Important Applications): To determine the amount of chlorine to be added to sample to achieve certain desired results, such as control of coliform densitites, destruction of certain chemical compounds, or establishment of specified chlorine residuals.

The method given here is applicable to drinking water, surface waters, domestic and industrial wastes, and saline water.

Summary of Method: A solution of known chlorine content is added incrementally to a series of sample aliquots. At the end of the stipulated contact time or when the desired result has been achieved the residual chlorine is measured by the appropriate method.

Limitations:

Range of Applicability: Not single, specific procedure, but varies with purpose or result to be achieved.

Pitfalls; Special Precautions: When the purpose is to obtain a specified chlorine residual, the same method of chlorine measurement should be used for operational control and laboratory testing.

All pertinent information should be included in report of results, such as: conditions of chlorination (pH, temperature, contact time); method used for determining the desired result; and the chlorine required to produce result.

Statistical Characteristics:

Accuracy and Precision: Nature of this test precludes the use of accuracy and precision statements.

Data Outputs: Not given, but assumed to be analog voltage displayed on meter or chart.

References:

1. Methods for Chemical Analysis of Water and Wastes. 1971. EPA National Environmental Research Center, Cincinnati, Ohio.

2. Standard Methods for Examination of Water and Wastewater. 1971. 13th Edition. American Public Health Association, Washington, D. C.

CHEMICAL OXYGEN DEMAND (COD)

Principal Detection Technique: Titration - (High Concentration)

Purpose of Measurement (Important Applications): To determine quantity of oxygen required to oxidize all organic matter in a wastewater sample under specified conditions.

Summary of Method: Organic substances in the sample are oxidized by potassium dichromate in 50% sulfuric acid solution at reflux temperature. Silver sulfate is used as a catalyst and mercuric sulfate is added to remove chloride interference. The excess dichromate is titrated with standard ferrous ammonium sulfate, using orthophenanthroline ferrous complex as an indicator.

Limitations:

Range of Applicability: Organic carbon concentration of 15 mg/l or higher.

Interferences: Chloride concentration over 2000 mg/l

Statistical Characteristics:

Precision: Eighty-nine analysts: Standard deviation of ±27.5 mg/l COD at known value 270 mg/l COD.

Special Sampling Requirements (Collection, Storage, Handling): Add 2 ml H_2SO_4 per liter of sample. Maximum storage time - 7 days.

References:

1. Methods for Chemical Analysis of Water and Wastes. 1971. EPA National Environmental Research Center, Cincinnati, Ohio.

2. Standard Methods for Examination of Water and Wastewater. 1971. 13th Edition. American Public Health Association, Washington, D. C.

CHEMICAL OXYGEN DEMAND (COD)

Principal Detection Technique: Titration - (Low Concentration)

Purpose of Measurement (Important Applications): Ambient surface water, domestic and industrial wastes with low oxygen demand characteristics.

Summary of Method: Similar to that for COD-High Level, except (1) that extreme care is exercised during sample acquisition and handling and during analysis to insure that no organic contaminants are introduced from glassware, atmosphere, etc., (2) that highly pure reagents are used, and (3) that chlorides are removed by complexing with mercuric sulfate.

Limitations:

Range of Applicability: 5 to 30 mg/l COD

Interferences: Organic contaminants; chlorides

Pitfalls; Special Precautions: Volatile materials may be lost during sulfuric acid addition step.

Calibration Requirements: Standardize reagents daily.

Special Sampling Requirements (Collection, Storage, Handling): Use glass sample bottles. Preserve with H_2SO_4. Test biologically active samples soon after acquisition.

References:

1. Methods for Chemical Analysis of Water and Wastes. 1971. EPA National Environmental Research Center, Cincinnati, Ohio.

2. Standard Methods for Examination of Water and Wastewater. 1971. 13th Edition. American Public Health Association, Washington, D. C.

CHEMICAL OXYGEN DEMAND (COD)

Principal Detection Technique: Titration - (Saline Waters)

Purpose of Measurement (Important Applications): COD Determination in saline waters.

Summary of Method: Organic and oxidizable inorganic substances in an aqueous sample are oxidized by potassium dichromate solution in 50 percent (by volume) sulfuric acid solution. The excess dichromate is titrated with standard ferrous ammonium sulfate using orthophenanthroline ferrous complex (ferroin) as an indicator. Mercuric sulfate is added to complex the chlorides during digestion.

Limitations:

Range of Applicability: Minimum of 250 mg/l COD when chloride concentration exceeds 1000 mg/l. (The removal of chlorides by $MgSO_4$ may not be complete in the case of strong brines.)

Interferences: Extraneous organic matter

Special Sampling Requirements (Collection, Storage, Handling): Use glass bottles if possible; preserve with H_2SO_4.

Reference:

1. Methods for Chemical Analysis of Water and Wastes. 1971. EPA National Environmental Research Center, Cincinnati, Ohio.

COLOR

Principal Detection Technique: Visual sensing

Purpose of Measurement (Important Applications): Measures color of water derived from naturally occurring materials, e.g. vegetable residues such as leaves, bark, humus, etc.

Summary of Method: Color is measured by visual comparison of the sample with platinum-cobalt standards. One unit of color is that produced by 1 mg/l platinum in the form of the chloroplatinate ion.

The Spectrophotometric and Tristimulus methods are useful for detecting specific color problems. The use of these methods, however, is laborious and unless determination of the hue, purity, and luminance is desired, they are of limited value.

Limitations:

Interferences: Turbidity; highly colored industrial wastes.

Pitfalls; Special Precautions: Biological activity may change color characteristics after sample is acquired.

Calibration Requirements: See Reference

Data Outputs: Usually a visual observation, manually recorded.

Special Sampling Requirements (Collection, Storage, Handling): Store sample at 4°C. Maximum holding time - 24 hours.

References:

1. Methods for Chemical Analysis of Water and Wastes. 1971. EPA National Environmental Research Center, Cincinnati, Ohio.

2. Standard Methods for Examination of Water and Wastewater. 1971. 13th Edition. American Public Health Association, Washington, D. C.

CYANIDE

Principal Detection Technique: Colorimetric

Purpose of Measurement (Important Applications): Ambient surface water, domestic and industrial wastes, saline waters.

Summary of Method: The cyanide as hydrocyanic acid (HCN) is released from metallic cyanide complex ions by means of a reflux-distillation operation and absorbed in a scrubber containing sodium hydroxide solution. The cyanide ion in the absorbing solution is then determined by volumetric titration or colorimetrically. The colorimetric measurement employs the pyridine-pyraxolone reaction in which the cyanide is coupled with free chlorine to form cyanogen chloride and then with the pyridine to a glutaconic aldehyde. The aldehyde then reacts with 1-phenyl-3-methyl-5-pyraxolone to form a highly colored blue dye.

Limitations:

Range of Applicability: Cyanide concentrations below 1 mg/1 (sensitive to about 0.5 ug/1).

Interferences: Sulfides, oxidizing substances.

Pitfalls; Special Precautions: Material tested may be highly toxic and should be treated accordingly. Sample should be maintained at a highly basic pH.

Statistical Characteristics:

Determination by 47 analysts yielded:

Precision:	Standard Deviation	Known CN Concentration of:
	0.020 mg/1	0.02 mg/1
	0.306 mg/1	1.10 mg/1

Data Outputs: Analog signal (meter)

Special Sampling Requirements (Collection, Storage, Handling):

- minimum sample size - 1 liter
- adjust pH to 11 at time of sample collection (using sodium hydroxide)
- analyze as soon as possible after collection (maximum holding time - 24 hours)
- store at 4°C.

References:

1. Methods for Chemical Analysis of Water and Wastes. 1971. EPA National Environmental Research Center, Cincinnati, Ohio.

CYANIDE

Principal Detection Technique: Titration

Purpose of Measurement (Important Applications): Ambient surface water, domestic and industrial wastes, saline waters.

Summary of Method: The cyanide as hydrocyanic acid (HCN) is released from metallic cyanide complex ions by means of a reflux-distillation operation and absorbed in a scrubber containing sodium hydroxide solution. The cyanide ion in the absorbing solution is then determined by volumetric titration or colorimetrically. The titrimetric measurement uses a standard solution of silver nitrate to titrate cyanide in the presence of a silver sensitive indicator.

Limitations:

Range of Applicability: Concentrations of cyanide exceeding 1 mg/l

Interferences: Sulfides, oxidizing substances

Pitfalls; Special Precautions: See summary for Cyanide Colorimetric

Statistical Characteristics:

Results by 47 analysts:

Precision:	Standard Deviation	Known CN Concentration of:
	0.035 mg/l	0.02 mg/l
	0.333 mg/l	1.10 mg/l

Data Outputs: Not stated. (Probably visual reading, recorded manually).

Special Sampling Requirements (Collection, Storage, Handling): See summary for Cyanide, Colorimetric.

Reference:

1. Methods for Chemical Analysis of Water and Wastes. 1971. EPA National Environmental Research Center, Cincinnati, Ohio.

DISSOLVED OXYGEN (DO)

Name of Measurement Method: Modified Winkler - Full Bottle Technique

Principal Detection Technique: Titration

Purpose of Measurement (Important Applications): Most ambient surface waters, most waste waters (but not domestic sewage).

Summary of Method: The sample is treated with manganous sulfate, potassium hydroxide and potassium iodide and finally sulfuric acid. The initial precipitate of manganous hydroxide combines with the dissolved oxygen in the sample to form a brown precipitate, manganic hydroxide. Upon acidification, the manganic hydroxide forms manganic sulfate which acts as an oxidizing agent to release free iodine from the potassium iodide. The iodine, which is stoichiometrically equivalent to the dissolved oxygen in the sample is then titrated.

Limitations:

Interferences: Oxidizing or reducing materials (especially sulfites, thiosulfates, polythionates, chlorine, hypochlorite) nitrate ions, ferrous ions, organic matter, high concentrations of suspended solids.

Pitfalls; Special Precautions: Sample may contain low concentrations of ferrous ions (less than 1 mg/l) and nitrates. High concentration of either interferes.

Statistical Characteristics: Exact data not available, the following are approximate.

Precision: Reproducibility of 0.2 mg/l of DO when known concentration is 7.5 mg/l DO.

Time of Measurement: Not stated, but a complex procedure.

Comments by Users: Most common interferences overcome by use of DO probe.

Data Outputs: Visual observation or analog signal.

Special Sampling Requirements (Collection, Storage, Handling): Grab sampling acquisition procedures are specified; also preservative reagent to be added. Analysis should be completed within 4-8 hours after acquisition of sample.

Reference:

1. Methods for Chemical Analysis of Water and Wastes. 1971. EPA National Environmental Research Center, Cincinnati, Ohio.

DISSOLVED OXYGEN (DO)

Name of Measurement Method: Probe Method

Principal Detection Technique: Electrometric

Purpose of Measurement (Important Applications): Ambient surface waters, domestic and industrial wastes.

Summary of Method: The most common instrumental probes for determination of dissolved oxygen in water are dependent upon electrochemical reactions. Under steady-state conditions, the current or potential can be correlated with DO concentrations. Interfacial dynamics at the probe-sample interface are a factor in probe response and a significant degree of interfacial turbulence is necessary. For precision performance, turbulence should be constant. The probe method may be used under any circumstances as a substitute for the modified Winkler procedure provided that the probe itself is standardized against the Winkler method on samples free of interfacing materials. The electronic readout meter for the output from dissolved oxygen probes is normally calibrated in convenient scale (0 to 10, 0 to 15, 0 to 20 mg/l, for example) with a sensitivity of approximately 0.05 mg/l.

Limitations:

Interferences: Sulfur compounds and certain reactive gases (e.g., chlorine) may interfere. Dissolved inorganic salts affect performance, but usually can be compensated for. pH variation interferences with some probes.

Pitfalls; Special Precautions: Probes may be sensitive to temperature and may require temperature compensation.

Statistaical Characteristics: Manufacturers' Claims:

Accuracy: ± 1% of true DO value

Precision: 0.1 mg/l repeatability

Time of Measurement: Not stated, but rapid.

Calibration Requirements: Not stated. (Should be calibrated against standards with approximately the same concentration of suspended solids as the sample).

Data Outputs: Electrical signal displayed on meter

Special Sampling Requirements (Collection, Storage, Handling): Not stated. Can provide continuous, in situ measurements.

Reference:

1. Methods for Chemical Analysis of Water and Wastes. 1971. EPA National Environmental Research Center, Cincinnati, Ohio.

FLUORIDE

Name of Measurement Method: Automated Complexone Method

Principal Detection Technique: Photometric, automated (Technicon AutoAnalyzer)

Purpose of Measurement (Important Applications): Ambient surface water, domestic and industrial wastes, saline waters.

Summary of Method: Fluoride reacts with red cerous chelate of alizarin complexone. A positive color is developed and intensity is measured by a colorimeter at about 650 nm. Technicon AutoAnalyzer is used.

Limitations:

Range of Applicability: 0.05 to 1.5 mg/l fluoride

Interferences: Aluminum

Statistical Characteristics:

Precision: At one laboratory, standard deviation of measurement was 0.018 mg/l F when actual concentrations were 0.06, 0.15, 0.55, and 1.08 mg/l.

Time of Measurement: 12 samples per hour after 30 minutes warming.

Data Outputs: Analog electrical signal recorded on stripchart.

Special Sampling Requirements (Collection, Storage, Handling): Not stated. Can provide continuous, *in situ* measurements.

Reference:

1. Methods for Chemical Analysis of Water and Wastes. 1971. EPA National Environmental Research Center, Cincinnati, Ohio.

HARDNESS

Principal Detection Technique: Titration

Purpose of Measurement (Important Applications): Drinking waters, ambient surface waters, domestic and industrial wastes.

Summary of Method: Calcium and magnesium ions in the sample are sequestered upon the addition of disodium dihydrogen ethylenediamine tetraacetate (Na-EDTA). The end point of the reaction is detected by means of Chrome Black T or Calmagite, which has a red color in the presence of calcium and magnesium and a blue color when the cations are sequestered.

Limitations:

Range of Applicability: All concentrations of hardness.

Interferences: Excess concentrations of heavy metals (may be removed by complexing with cyanide)

Statistical Characteristics: 43 analysts in 17 laboratories obtained the following results:

Accuracy (Bias)	Precision (Std. Dev.)	Over Known Range of Hardness as $CaCO_3$
-0.003 mg/l (as $CaCO_3$)	2.87 mg/l	31 mg/l
-0.24	2.52	33
0.4	4.87	182
-2.0	2.98	194
-13.0	9.65	417
-14.3	8.73	444

Data Outputs: Not stated. Assume visual observation, manual recording.

Special Sampling Requirements (Collection, Storage, Handling): None. Maximum holding time, 7 days.

References:

1. Methods for Chemical Analysis of Water and Wastes. 1971. EPA National Environmental Research Center, Cincinnati, Ohio.

TOTAL HARDNESS

Name of Measurement Method: Automated Method

Principal Detection Technique: Colorimetry

Purpose of Measurement (Important Applications): Surface waters and saline waters

Summary of Method: The disodium magnesium EDTA exchanges magnesium on an equivalent basis for any calcium and/or other cations to form a more stable EDTA chelate than magnesium. The free magnesium reacts with calmagite at a pH of 10 to give a red-violet complex. Thus, by measuring only magnesium concentration in the final reaction stream, an accurate measurement of total hardness is possible.

Method assumes the use of a Technicon AutoAnalyzer. Colorimeter is equipped with 520 nm filter.

Limitations:

Range of Applicability: 10 to 400 mg/l expressed as $CaCO_3$

Statistical Characteristics:

Precision: Results from a single laboratory:

Std. Dev.	Known Concentration
± 1.5 mg/l	19 mg/l $CaCO_3$
± 1.5	120
± 4.5	385
± 5.0	366

Time of Measurement:
12 determinations per hour. 30 minutes warm up.

Calibration Requirements: Apparatus must be calibrated against stock solutions of known concentration.

Data Outputs: Analog electrical signal, recorded on stripchart.

References:

1. Methods for Chemical Analysis of Water and Wastes. 1971. EPA National Environmental Research Center, Cincinnati, Ohio.

2. Technicon AutoAnalyzer Methodology. 1960. Bulletin No. 2, Channcey, New York.

METALS

Name of Measurement Method: Atomic Absorption

Parameter(s) Measured: Metals

Aluminum
Arsenic
Cadmium
Calcium
Chromium
Copper
Iron
Lead
Magnesium
Manganese
Potassium
Silver
Sodium
Zinc

Principal Detection Technique: Atomic Absorption Spectroscopy.

Purpose of Measurement (Important Applications): Rapid determination of certain metals in ambient surface waters, domestic and industrial water, and saline waters.

Summary of Method: Atomic absorption spectroscopy is similar to flame emission photometry in that a sample is atomized and aspirated into a flame. Flame photometry, however, measures the amount of light emitted, whereas, in atomic absorption spectrophotometry, a light beam is directed through the flame into a monochromator, and onto a detector that measures the amount of light absorbed. In many instances absorption is more sensitive because it depends upon the presence of free, unexcited atoms and generally the ratio of unexcited to excited atoms at a given moment is very high. Since the wavelength of the light beam is characteristic of only the metal being determined, the light energy absorbed by the flame is a measure of the concentration of that metal in the sample. This principle is the basis of atomic absorption spectroscopy.

Analytical procedures specific to each of the metals listed above are given in the Reference cited.

Limitations:

Range of Applicability: Detection limits, sensitivities, and optimum ranges of concentrations, vary with make and model of atomic absorption spectrometer. The following table provides some indication of ranges of measurement. In many cases the range can be extended higher or lower by instrument adjustment, use of a different wavelength, or sample pretreatment.

Metal	Detection Limit (mg/l)	Sensitivity (mg/l)	Optimum Concentration Range (mg/l)
Aluminum	0.1	0.4	10 - 1000
Arsenic	0.05	1.0	10 - 100
Cadmium	0.001	0.004	0.1 - 2
Calcium	0.003	0.07	1 - 200
Chromium	0.01	0.02	1 - 200
Copper	0.005	0.04	0.1 - 10
Iron	0.004	0.006	0.1 - 20
Lead	0.01	0.06	1 - 10
Magnesium	0.0005	0.005	0.01 - 2
Manganese	0.005	0.04	0.1 - 20
Potassium	0.005	0.01	0.01 - 2
Silver	0.01	0.05	0.1 - 20
Sodium	0.001	0.003	1 - 200
Zinc	0.005	0.02	0.1 - 2

Accuracy and Precision Data:

Metal	Metal Concentration (ug/l)	Relative Error (Percent)	Relative Standard Deviation (Percent)
Direct Determination			
Cadmium	50	8.15	21.62
Chromium	50	2.29	26.44
Copper	1000	3.42	11.23
Iron	300	0.64	16.53
Magnesium	200	6.30	10.49
Manganese	50	6.00	13.50
Silver	50	10.57	17.47
Zinc	500	0.41	8.15
Extracted Samples			
Cadmium	10	3.03	72.77
Lead	50	19.00	23.46

Time of Measurement: Rapid

Calibration Requirements: See Reference

Data Outputs: Electrical signals

Special Sampling Requirements (Collection, Storage, Handling):
See Reference

References:

1. Methods for Chemical Analysis of Water and Wastes. 1971. EPA National Environmental Research Center, Cincinnati, Ohio.

MERCURY

Name of Measurement Method: Flameless Atomic Absorption

Principal Detection Technique: Atomic Absorption Spectroscopy

Purpose of Measurement (Important Applications): Ambient surface waters, saline waters, wastewaters, and effluents. May also be used for fish tissue, mud, sediments, and other materials following proper digestion.

Summary of Method: The flameless AA procedure is a physical method based on the absorption of radiation at 253.7 nm by mercury vapor. The mercury is reduced to the elemental state and aerated from solution in a closed system. The mercury vapor passes through a cell positioned in the light path of an atomic absorption spectrophotometer. Absorbance (peak height) is measured as a function of mercury concentration and recorded on a stripchart.

Limitations:

Range of Applicability: Detection limit is 0.2 ug/l mercury

Interferences: Sulfides, copper, high concentration of chlorides, and certain volatile organics

Statistical Characteristics: Using an Ohio River composite sample with a background mercury concentration of 0.35 ug/l, spiked with concentrations of 1, 3, and 4 ug/l, the standard deviations were ±0.14, ±0.10 and ±0.08 ug/l respectively. Standard deviation at the 0.35 level was 0.16. Percent recoveries at the three levels were 89, 87, and 87% respectively.

Calibration Requirements: Detailed calibration instructions included in Reference below.

Data Outputs: Analog electrical signal, displayed on stripchart.

Special Sampling Requirements (Collection, Storage, Handling): Acidify sample to pH of 2 or lower.

Reference:

1. Methods for Chemical Analysis of Water and Wastes. 1971. EPA National Environmental Research Center, Cincinnati, Ohio.

MERCURY

Name of Measurement Method: Cold Vapor Technique - (Biological Materials)

Medium: Fish tissue (and other biological materials)

Principal Detection Technique: Atomic absorption spectroscopy

Purpose of Measurement (Important Applications): To measure total mercury (organic and inorganic) in fish and in other biological materials.

Summary of Method: Weighed portion of sample is digested with sulfuric and nitric acid and oxidized overnight with potassium permanganate. Mercury content is measured by the conventional cold vapor (Flameless AA) technique summarized in Method Summary for Mercury in Water (this compendium) and described in References (1) and (2) of that Summary.

Limitations:

Range of Applicability: 0.2 to 5 ug/g. (May be extended by varying sample size or through instrument control).

Interferences: Most interferences are destroyed during digestion and oxidation steps.

Pitfalls; Special Precautions: See "Sampling Requirements" below.

Statistical Characteristics:

Precision: The following standard deviations on replicate fish samples were recorded at the indicated levels: 0.19 ug/g±0.01; 0.74 ug/g±0.05; and 2.1 ug/g±0.07. The coefficients of variation at these levels were 11.9 percent, 7 percent, and 3.6 percent respectively.

Time of Measurement: Several hours.

Data Outputs: Electrical signals displayed or recorded.

Special Sampling Requirements (Collection, Storage, Handling): Mercury is not uniformly distributed throughout the whole fish and it is therefore necessary to decide in advance which part of the fish is to be analyzed. In the case of a large specimen, only a selected part of the fish may be examined. (Ordinarily, only the edible flesh is analyzed). In any event, the portion analyzed should be reported.

If it becomes necessary to freeze the fish before analysis, the sample should not be allowed to thaw before weighing as higher results may be observed.

References:

1. Mercury in Fish. 1972. Method Description received from Analytical Quality Control Laboratory, EPA National Environmental Research Center, Cincinnati, Ohio.

2. Uthe, J.F., F.A.J. Armstrong and M.P. Stainton. Mercury Determination in Fish Samples by Wet Digestion and Flameless Atomic Absoprtion Spectrophotometry. Jour. Fisheries Research Board of Canada, 27:805.

MERCURY

Name of Measurement Method: Gas Chromatography (In Fish)

Medium: Fish Tissue

Purpose of Measurement (Important Applications): To determine concentration of methyl mercury in fish samples of all types.

Summary of Method: A measured weight of fish is treated with acid and bromide salt. Methyl mercury is extracted as methyl mercury bromide with toluene. A cleanup operation is performed by first extracting the toluene layer with aqueous solution of sodium thiosulfate. The methyl mercury thiosulfate complex formed in the aqueous layer is then reacted with an excess of potassium iodide. Benzene is used to extract any methyl mercury as the iodide salt. A portion of the benzene extract is chromatographed directly and response is compared to standard responses. The iodide is detected using gas chromatography with an electron capture detector.

Limitations:

Sensitivity: 0.01 ug/g

Interferences: Materials in sample will generally not cause a problem. Toluene from extraction not removed by cleanup step may interfere.

Pitfalls; Special Precautions: Potassium iodide solution decomposes readily. Any free iodine may cause interfering peaks on chromatogram.

Statistical Characteristics:

Accuracy and Precision: When seven portions of a filet of a large white perch were analyzed by this method, a mean value of 0.37 ug/g was obtained, with a standard deviation of 0.034 ug/g.

Recoveries of methyl mercury chloride injected into this same fish at the 0.20 ug/g level averaged 95.5 percent.

Time of Measurement: Approximately one hour.

Calibration Requirements: No unusual requirements. Calibration procedure given in Reference.

Data Outputs: Analog electrical signal recorded as chromatogram.

Special Sampling Requirements (Collection, Storage, Handling): Analysis should be performed on the fish before decay has begun. Fresh or frozen fish can be analyzed with confidence; results on partially decomposed samples must be viewed with caution and reported as such.

Fish samples can dehydrate rapidly unless protected during handling. Defrosting and refreezing a frozen sample before analysis, and permitting a sample to stand open at room temperature before weighing must be avoided.

Selection of portions of the fish must be of a consistent nature and properly identified as to location on the specimen.

References:

1. Methyl Mercury in Fish. 1972. Provision Method Description received from Analytical Quality Control Laboratory, EPA National Environmental Research Center, Cincinnati, Ohio.

MERCURY

Medium: Sediment

Name of Measurement Method: Gas Chromatography (In Sediment)

Principal Detection Technique: Electron capture gas chromatography

Purpose of Measurement (Important Applications).: This method is applicable to bottom samples such as mud, sludge, silt and gravel.

Summary of Method: A measured weight of sediment is treated with acid and bromide salt. Methyl mercury is extracted with toluene as methyl mercury bromide. A cleanup operation is performed by first extracting the toluene layer with an aqueous solution of sodium thiosulfate. The methyl mercury thiosulfate complex formed in the aqueous layer is then reacted with an excess of potassium iodide. Benzene is used to extract any methyl mercury as the iodide salt. A portion of the benzene extract is chromatographed directly and response is compared to standard responses. The iodide is detected using gas chromatography with an electron capture detector.

Limitations:

Sensitivity: 0.001 ug/g

Interferences: Materials in sample generally will not cause a problem.

Pitfalls; Special Precautions: Potassium iodide solution may decompose to free iodine, which may cause interfering peaks on the chromatogram.

Statistical Characteristics:

Accuracy and Precision: Ten dfferent sediment samples with a background level of less than 0.0005 ug/g were spiked with 0.010 ug/g and measured with this test. The average was 0.00963 ug/g and the standard deviation was 0.00087 ug/g.

Calibration Requirements: Given in Reference below

Data Outputs: Analog electrical signal recorded as chromatogram.

Special Sampling Requirement (Collection, Storage, Handling): It should be understood that methyl mercury is generated from inorganic mercury by biological methylation. Since this can occur at widely varying rates in samples stored in a laboratory, analyses intented to determine the methyl mercury concentration existing at the sampling site at the time of sampling should be performed as quickly as possible. Storing samples frozen or refrigerated before analysis is acceptable but is a less desirable alternative.

References:

1. Methyl Mercury in Sediment. 1972. Provision Method. Description received from Analytical Quality Control Laboratory, EPA National Environmental Research Center, Cincinnati, Ohio.

ATOMIC ABSORPTION SPECTROSCOPY

Name of Measurement Method: Mercury -- Cold Vapor Technique

Purpose of Measurement (Important Applications): This method is applicable to surface waters, saline waters, wastewaters, effluent and domestic sewage.

Summary of Method: The atomic absorption procedure is a physical method based on the absorption of radiation at 253.7 nm by mercury vapor. The mercury is reduced to the elemental state and aerated from solution in a closed system. The mercury vapor passes through a cell positioned in the light path of an atomic absorption spectrophotometer. (Instruments designed specifically for the measurement of mercury using the cold vapor technique are commercially available and may be substituted for the atomic absorption spectrophotometer). Absorbance (peak height) is measured as a function of mercury concentration and recorded in the usual manner.

In addition, inorganic forms of mercury, organic mercurials may also be present in an effluent or surface water sample. The organomercury compounds will not respond to the flameless atomic absorption techniques unless they are first broken down and converted to mercuric ions. Potassium permaganate oxidizes many of these compounds but recent studies have shown that a number of organic mercurials, including phenyl mercuric acetate and methyl mercuric chloride, are only partially oxidized by this reagent. Potassuim persulfate has been found to give approximately 100 percent recovery when used as the oxidant with these compounds. Therefore, a persulfate oxidation step following the addition of the permanganate has been included to insure that organomercury compounds, if present, will be oxidized to the mercuric ion before measurement. A heat step is required for methyl mercuric chloride when present in or spiked to a natural system. For distilled water the heat step is not necessary.

Limitations:

Range of Applicability: The range of the method may be varied through instrument and/or recorder expansion. Using a 100 ml sample, a detection limit of 0.2 ug Hg/l can be achieved; concentrations below this level should be reported as 0.2.

Interferences: Sulfides (interference can be eliminated by addition of potassium permaganate), high concentrations of copper and chlorides. (Certain volatile organic materials may also interfere.)

Statistical Characteristics:

Accuracy and Precision: Using an Ohio River composite sample with a background mercury concentration of 0.35 ug/l, spiked with concentrations of 1, 3 and 4 ug/l, the standard deviations were ±0.14, ±0.10 and ±0.08 ug/l, respectively. Standard deviation at the 0.35 level was ±0.16. Percent recoveries at the three levels were 89, 87, and 87 percent, respectively.

Time of Measurement: Several hours.

Calibration Requirements: No unusual requirements. See Reference for detailed procedure.

Special Sampling Requirements (Collection, Storage, Handling): Acidify sample with nitric acid to pH of 2 or lower immediately upon collection. (If only dissolved mercury is to be determined, sample should be filtered before acidification.)

References:

1. Mercury in Water. 1972. Modification of procedure appearing in the 1971 issue of Methods for Chemical Analysis of Water and Wastes, Analytical Quality Control Laboratory, Cincinnati, Ohio.

METHYLENE BLUE ACTIVE SUBSTANCES (MBAS)

Name of Measurement Method: Methylene Blue Method

Principal Detection Technique: Colorimetry

Purpose of Measurement (Important Applications): Determination of concentration of detergents, phosphates, surfactants in drinking water, ambient surface waters, domestic and industrial wastes.

Summary of Method: The dye, methylene blue, in aqueous solution reacts with anionic-type surface active materials to form a blue colored salt. The salt is extractable with chloroform and the intensity of color produced is proportional to the concentration of MBAS.

Limitations:

Range of Applicability: 0.025 to 100 mg/l of MBAS (expressed as linear alkyl sulfonate - LAS)

Interferences: Chloride at concentrations over 1,000 mg/l sulfonates, carboxylates, phosphates, phenol, cyanates, and thiocynaides (at concentrations higher than normally encountered in water or wastewater).

Statistical Characteristics:

Accuracy: +1.2% to -11% bias (conditions as given for Precision).

Precision: Determinations by 110 analysts exhibited relative standard deviations of 10 to 15 percent for known concentrations of MBAS ranging from 0.27 to 2.94 mg/l of LAS

Data Outputs: Analog electrical signal, displayed on meter.

References: Methods for Chemical Analysis of Water and Wastes, EPA National Environmental Research Center, Cincinnati, Ohio (1971).

NITROGEN

Name of Measurement Method: Ammonia Distillation Procedure

Principal Dectection Technique: Colorimetry

Purpose of Measurement (Important Applications): Ambient surface waters, domestic and industrial wastewaters, and saline waters.

Summary of Method: The sample is buffered at pH of 9.5 with a borate buffer in order to decrease hydrolysis of cyanates and organic nitrogen compounds, and is then distilled into a solution of boric acid. The ammonia in the distillate can be determined either colorimetrically by nesslerization, or titrimetrically with standard sulfuric acid with the use of a mixed indicator, the choice between these two procedures depending on the concentration of the ammonia.

Limitations:

Range of Applicability: 0.05 to 1.0 mg/l ammoniacal nitrogen

Interferences: Cyanates, certain alcohols, aldehydes, and ketones may interfere. (Certain amines would interfere if distillation step were not included.

Pitfalls; Special Precautions: Residual chlorine must be removed. If sample is preserved by a mercury salt, the mercury must be complexed.

Statistical Characteristics: 24 analysts in 16 laboratories obtained:

Known Concentration in mg/l Nitrogen	Accuracy (Bias) mg/l N	Precision (Std. Dev.) mg/l N
0.21	-0.01	0.122
0.26	-0.05	0.070
1.71	+0.01	0.244
1.92	-0.04	0.279

Calibration Requirements: Set up a series of standards in Nessler tubes. Care needed in calibrating for saline water tests.

Data Outputs: Meter reading (analog electrical signal)

Special Sampling Requirements (Collection, Storage, Handling): Preserve with mercuric chloride and store at 4° C.

References:

1. Methods for Chemical Analysis of Water and Wastes. 1971. EPA National Environmental Research Center, Cincinnati, Ohio.

NITROGEN

Name of Measurement Method: Ammonia

Principal Detection Technique: Titrimetric

Purpose of Measurement (Important Applications): Surface waters, domestic and industrial wastewaters, and saline waters.

Summary of Method: The sample is buffered at pH of 9.5 with borate buffer in order to decrease hydrolysis of cynates and organic nitrogen ammonia in the distillate can be determined either coloimetrically by nesslerization or titrimtrically with standard sulfuric acid and the use of a mixed indicator, the choice between these two procedures depending on the concentration of the ammonia.

Limitations:

Range of Applicability: 1.0 to 25 mg/l ammoniacal nitrogen.

Interferences: Cyanates, hydrozine and similar compounds.

Pitfalls; Special Precautions: Residual chlorine and mercury must be removed.

Sampling Sampling Requirements (Collection, Storage, Handling): Preserve with mercuric chloride and store at 4° C.

References:

1. Methods for Chemical Analysis of Water and Wastes. 1971. EPA National Environmental Research Center, Cincinnati, Ohio.

NITROGEN

Name of Measurement Method: Ammonia

Principal Detection Method: Automatic Colorimetric

Purpose of Measurement (Important Applications): Surface Waters, saline waters.

Summary of Method: The intensity of the indophenol blue color, formed by the reaction of ammonia with alkaline phenol hypochlorite, is measured. Sodium nitroprusside is used to intensify the blue color.

Uses Technicon AutoAnalyzer. Colorimeter is equipped with 360 or 650 filters.

Limitation:

Range of Applicability: .01 to 2.0 mg/l nitrogen as NH_3

Pitfalls; Special Precautions: Marked variations in pH among samples should be eliminated.

Statistical Characteristics:

Precision: In a single laboratory using surface water at water at concentrations of 1.41, 0.77, 0.59, 0.43 mg/l of NH_3-N, the standard deviation was ±.005 mg/l.

Calibration Requirements: Requires calibration against samples, both for fresh water and substitute ocean water of specified constituency.

Data Outputs: Analog electrical signal displayed on stripchart

Special Sampling Requirements (Collection, Storage, Handling): Preserve with mercuric chloride and refrigerate at 40°. Max. holding time - 7 days

References:

1. Methods for Chemical Analysis of Water and Wastes. 1971. EPA National Environmental Research Center, Cincinnati, Ohio.

NITROGEN

Name of Measurement Method: Kjeldahl

Principal Detection Technique: Tritrimetric

Purpose of Measurement (Important Applications): Surface waters, domestic and industrial wastes, saline waters.

Summary of Method: Total Kjeldahl nitrogen is defined as the sum of the free ammonia and organic nitrogen compounds which are converted to ammonium sulfate under defined conditions of digestion. The procedure converts nitrogen components of biological orgin such as amino acids, peptides, and proteins to ammonia, but may not convert the nitrogenous compounds of certain industrial wastes such as amines, hydrazones, nitro compounds, and others.

The sample is heated in the presence of concentrated sulfuric acid, H_2SO_4) and $HgSO_4$ and evaporated until SO_2 fumes are obtained and the solution becomes colorless or pale yellow. The residue is collected, diluted, and is treated and made alkaline with a hydroxide-thiosulfate solution. The ammonia is distilled and determined after distillation either by nesslerization or titrimetrically.

Limitations:

Range of Applicability: Concentration above 1 mg/l of nitrogen.

Statistical Characteristics: Results obtained by 31 analysts in 20 laboratories

Known Conc. of Nitrogen, Kjeldahl mg N/liter	Precision as Standard Deviation mg N/liter	Accuracy as Bias, mg N/liter	Bias,
0.20	0.197	+15.54	+.03
0.31	0.247	+ 5.45	+.02
4.10	1.056	+ 1.03	+.04
4.61	1.191	- 1.67	-.08

Special Sampling Requirements (Collection, Storage, Handling): Unstable. Analyze soon after sample is acquired.

References:

1. Methods for Chemical Analysis of Water and Wastes. 1971. EPA National Environmental Research Center, Cincinnati, Ohio.

NITROGEN

Name of Measurement Method: Kjeldahl

Principal Detection Technique: Automated Phenolate

Purpose of Measurement (Important Applications): Ambient surface waters, saline waters, domestic and industrial wastes.

Summary of Method: The sample is automatically digested with a sulfuric acid solution containing potassium sulfate and mercuric as a catalyst to convert organic nitrogen to ammonium sulfate. The solution is then automatically neutralized with sodium hydroxide solution and treated with alkaline phenol reagent and sodium hypochlorite reagent. This treatment forms a blue color designated as indophenol. Sodium nitroprusside, which increases the intensity of the color, is added to obtain necessary sensitivity for measurement of low level nitrogen.

Utilizes Technicon AutoAnalyzer. A colorimeter with 630 nm filter is used.

Limitations:

Range of Applicability: Nitrogen concentrations from 0.05 to 2.0 mg/l

Interferences: Iron, chromium, or copper ions may interfere

Statistical Characteristics: Results from six laboratories analyzing natural waters with known concentrations of Kjeldahl nitrogen:

Known Conc. of Kjeldahl-Nitrogen mg N/liter	Precision as Standard Deviation Kjeldahl-N, mg N/liter	Accuracy as Bias %	Accuracy as Bias mg N/liter
1.89	0.54	-24.6	- .46
2.18	0.61	-28.3	- .62
5.09	1.25	-23.8	-1.21
5.81	1.85	-21.9	-1.27

Time of Measurement: About 20 samples per hour

Calibration Requirement: Requires preparation of standardizations

Data Outputs: Analog electrical signal recorded on stripchart

Special Sampling Requirements (collection, storage, handling): Preserve with mercuric chloride; refrigerate at 4°C. Analyze immediately after acquistion.

References:

1. Methods for Chemical Analysis of Water and Wastes. 1971. EPA National Environmental Research Center, Cincinnati, Ohio.

NITROGEN

Name of Measurement Method: Kjeldahl

Principal Detection Technique: Colorimetric

Purpose of Measurement (Important Applications): Ambient surface waters, saline waters, domestic and industrial wastes.

Summary of Method: See Summary for Nitrogen, Kjeldahl, Total, Colorimetric. For Nesslerization, a spectrophometer or colorimeter is used, filtered at 400 to 525 nm.

Limitations:

Range of Applicability: Concentrations of nitrogen below 1 mg/l

Statistical Characteristics: See Method Summary for Nitrogen, Kjeldahl, Total (Titrimetric)

Data Outputs: Meter reading (analog electrical signal), recorded manually.

Special Sampling Requirements (Collection, Storage, Handling): Unstable, analyze immediately after sample is acquired.

References:

1. Methods for Chemical Analysis of Water and Wastes. 1971. EPA National Environmental Research Center, Cincinnati, Ohio.

NITROGEN

Principal Detection Technique: Nitrite

Purpose of Measurement (Important Applications): Ambient surface waters, saline waters, domestic and industrial wastes.

Summary of Method: The diazonium compound formed by diazotation of sulfanilamide by nitrite in water under acid conditions is coupled with N-(1-naphtyl)-ethylenediamine to produce a reddish-purple color which is read in a spectrophotometer at 540nm against a blank. Concentration of NO_2-N is plotted against optical density.

Limitations:

Range of Applicability: 0.05 to 1.0 mg/l nitrite nitrogen

Interferences: Strong oxidizing or reducing agents or high alkalinity

Calibration Requirements: Requires use of standard solutions

Data Outputs: Meter reading (analog voltage)

Special Sampling Requirements (Collection, Storage, Handling): Preserve with mercuric chloride; refrigerate at 4½C. Maximum storage time - 7 days.

References:

1. Methods of Chemical Analysis of Water and Wastes. 1971. EPA National Environmental Research Center, Cincinnati, Ohio.

NITROGEN

Name of Measurement Method: Nitrate-Nitrite

Principal Detection Technique: Automated Cadmium Reduction Method

Purpose of Measurement (Important Applications): Determines nitrates and nitrites, singly or combined, in surface and saline waters.

Summary of Method: The initial step is to reduce the nitrates to nitrites by using a cadmium-copper catalyst. The nitrites (those orginally present plus reduced nitrates) are then reacted with sulfanilamide to form the diazo compound which is then coupled in an acid solution (pH 2.0-2.5) with N-1 naphtylethylenediamine hydrochloride to form the azo dye. The azo values are readily obtainable by carrying out the procedure--first with, and then without, the initial Cd-Cu reduction step.

A Technicon AutoAnalyzer is used. The colorimeter is equipped with 540 nm filters.

Limitations:

Range of Applicability: 0.05 to 10.0 mg/l nitrogen present as nitrate.

Interferences: Ammonia and primary amines; some metal ions (mercury and copper) may produce interfering color complexes.

Statistical Charateristics: Three laboratories analyzed four natural water samples containing exact concentrations of inorganic nitrate, with the following results:

Known Conc. of Nitrate Nitrogen mg N/liter	Precision as Standard Deviation mg N/liter	Accuracy as Bias, %	Bias, mg N/liter
0.29	0.012	+ 5.75	+.017
0.35	0.092	+18.10	+.063
2.31	0.318	+ 4.47	+.103
2.48	0.176	- 2.69	-.067

Time of Measurement: Not stated, but assumed to be rapid (i.e., several samples per hour.

Calibration Requirements: Calibration against standard solution is necessary. Analysis of saline waters requires calibration against substitute ocean water.

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Data Outputs: Analog electrical signal displayed on stripchart.

Special Sampling Requirements (collection, storage, handling): Sample is not stable since amines in natural waters may react with nitrites. Sample should be analyzed as soon as possible after acquisition.

References:

1. Methods for Chemical Analysis of Water and Wastes. 1971. EPA National Environmental Research Center, Cincinnati, Ohio.

NITROGEN

Name of Measurement Method: Nitrate and Nitrite

Principal Detection Technique: Automated Hydrazine Reduction Method

Purpose of Measurement (Important Applications): Ambient surface waters and domestic or industrial wastes.

Summary of Method: This method, using the Technicon AutoAnalyzer, determines NO_2-N by the conventional diazotization-coupling reaction. The NO_3-N is reduced with hydrazine sulfate in another portion of the sample and the nitrite thus formed is determined in the usual manner.

Subtraction of the NO_2-N orginally present in the sample from the total NO_2-N will give the orginal NO_3-N concentration in terms of NO_2-N.

Utilizes Technicon AutoAnalyzer.

Limitations:

Range of Applicability: Nitrite or nitrate nitrogen in concentrations of 0.05 to 10.0 mg/liter.

Interferences: The following substances may interfere (concentration below which interference will not occur are listed in Reference): chlorides, phosphates, sulfides, ammonical nitrogen, manganese, calcium, and ferric ions, and ABS.

Pitfalls; Special Precautions: Toxic reagents

Statistical Characteristics: Nine laboratories analyzed four natural water samples containing exact concentrations of inorganic nitrate, with the following results:

Known Conc. of Nitrate Nitrogen mg N/liter	Precision as Standard Deviation mg N/liter	Accuracy as Bias, %	Accuracy as Bias, mg N/liter
0.29	0.053	-0.8	.002
0.35	0.058	+1.9	.007
2.31	0.258	+3.0	.07
2.48	0.217	-1.2	.03

In a single laboratory, using surface water samples at concentrations of 0.1, 0.2, 0.8, and 2.1 mg-N/1, the standard deviations were ±0.04, ±0.05, and ±0.05, respectively.

Time of Measurement: About 20 samples per hour

Calibration Requirements: See Reference.

Data Outputs: Analog electrical signal displayed on stripchart.

Special Sampling Requirements (collection, storage, handling): Preserve sample with mercuric chloride. Refrigerate at 4°C. Maximum holding time - 7 days.

References:

1. Methods for Chemical Analysis of Water and Wastes. 1971. EPA National Environmental Research Center, Cincinnati, Ohio.

NITROGEN

Name of Measurement Method: Organic Plus Ammonia

Principal Detection Technique: Automated Phenolate Method

Purpose of Measurement (Important Application): Ambient surface waters and saline waters.

Summary of Method: Organic nitrogen is determined by manually digesting the sample with potassium persulfate and sulfuric acid to convert the organic nitrogen, and any ammonia present, to ammonium sulfate. Subsequently, the automated phenol-hypochlorite procedure is used to measure the ammonia nitrogen. Nitrate-nitrite nitrogen is not measured by this procedure.

Utilized Technicon AutoAnalyzer with colorimeter equipped with 650 nm filter.

Limitations:

Range of Applicability: Nitrogen concentrations of 1.0 to 10.0 mg/l

Time of Measurement: Not stable. Estimated to be between one and two hours per determination.

Calibration Requirements: Calibrate against standard solutions

Data Outputs: Analog electrical signal recorded on stripchart.

Special Sampling Requirements (collection,storage,handling): Preserve with mercuric chloride. Refrigerate at 4½C. Maximum storage time - 7 days.

Reference:

1. Methods for Chemical Analysis of Water and Wastes. 1971. EPA National Environmental Research Center, Cincinnati, Ohio.

TRISODIUM - NITRILOTRIACETIC ACID (NTA)

Name of MeasurementMethod: Zinc-Zincon Method

Principal Detection Technique: Colorimetry

Purpose of Measurement (Important Applications) Ambient surface waters (non-saline).

Summary of Method: Zinc forms a blue-colored complex with 2 carboxy-2'-hydroxy-5'-sulfoformazylbenzene (Zincon) in a solution buffered to pH 9.2. When NTA is added, the Zn-Zincon complex is broken which reduces the optical density in proportion of the amount of NTA present.

A photometer equipped with 620 nm filter is used.

Limitations:

Range of Applicability: 0.5 to 10.0 mg/l NTA

Interferences: Cations of common metals (calcium, magnesium, zinc, copper iron, manganese, et al).

Pitfalls; Special Precautions: Most interfering cations can be removed by ion-exchange resin.

Statistical Characteristics:

Precision: In a single laboratory, using spiked surface water samples at concentrations of 0.5, 2, 6, and 10 mg/l NTA, standard deviations were ±0.17, ±0.14, ±0.1, and ±0.16, respectively.

Calibration Requirements: Require stock solution

Data Outputs: Analog electrical signal displayed on meter.

Special Sampling Requirements (collection storage, handling): Sample should be analyzed as soon as possible after acquistion, since NTA is biodegradable.

References:

1. Methods for Chemical Analysis of Water and Wastes. 1971. EPA National Environmental Research Center, Cincinnati, Ohio.

TRISODIUM-NITRILOTRIACETIC ACID (NTA)

Name of Measurement Method: Automated Zinc-Zincon Method

Principal Detection Technique: Colorimetry

Purpose of Measurement (Important Applications): Ambient surface waters (non-saline).

Summary of Method: Zinc forms a blue-colored complex with 2-carboxy-2'-hydroxy-5'sulfoformazylbenzene (Zincon) in a solution buffered to pH 9.2. When NTA is added, the Zn-Zincon complex is broken which reduces the optical density in proportion to the amount of NTA present.

A Tehcnicon AutoAnalyzer is used. The colorimeter is equipped with 600 to 625 nm filter.

Limitations:

Range of Applicability: 0.04 to 1.0 mg/l or 0.5 to 10.0 mg/l NTA, depending on type of manifold system.

Interferences: Common cations (calcium, magnesium, copper iron, mangagnese). Constituents of sewage cause some interference also.

Pitfalls; Special Precautions: Not applicable to saline water.

Statistical Characteristics:

Reproducibility: In a single laboratory, using surface water samples at concentrations of 0.1, 0.18, 0.27, and 0.44 mg/l, the standard deviations were ±0.01, ±0.004, ±0.005, respectively. At concentrations of 1.3, 4.0, 5.8, and 7.4 mg/l, the standard deviations were ±0.05, ±0.05, ±0.07, and ±0.1, respectively.

Time of Measurement: About 13 samples per hour.

Calibration Requirements: Requires standard solutions.

Data Outputs: Analog electric signal, recorded automatically.

Special Sampling Requirements Collection, Storage, Handling): Analyze soon after sample collection because of biodegradability of NTA.

Reference:

1. Methods for Chemical Analysis of Water and Wastes. 1971. EPA National Environmental Research Center, Cincinnati, Ohio.

PHOSPHORUS

Name of Measurement Method: Single Reagent Method

Principal Detection Technique: Colorimetry

Purpose of Measurement (Important Applications): Ambient surface waters, drinking water, domestic and industrial wastes, saline waters (may also be applicable to sediments, sludges, and algae blooms).

Summary of Method: Ammonium molybdate and potassium antimonyl-tartrate react in an acid medium with dilute solutions of phosphorus to form an antimony-phospho-molybdate complex. This complex is reduced to an intensely blue-colored complex by ascorbic acid. This color is proportional to the phosphorus concentration.

Only orthophosphate forms a blue color in this test. Polyphosphates (and some organic-phosphorus compounds) may be converted to the orthophosphate form by sulfuric-acid-hydrolysis. Organic phosphorus compounds may be converted to the orthophosphate form by sulfuric acid hydrolysis. Organic phosphorus compounds may be converted to the orthophosphate form by persulfate digestion.

A spectrophotometer of filter photometer suitable for measurements at 880 nm is used.

Limitations:

Range of Applicability: 0.01 to 0.5 mg/l phosphorus

Interferences: High concentrations of iron. Also, mercuric chloride (when used as a preservative).

Pitfalls; Special Precuations: Avoid use of commercial detergents for cleaning analyzed natural water samples containing exact concentrations of organic phosphate, with the following results:

Known Conc. of Total Phosphorus mg P/liter	Precision as Standard Deviation mg P/liter	Bias, %	Accuracy as Bias, mg P/liter
0.110	0.033	+ 3.091	+.003
0.132	0.051	+11.99	+.016
0.772	0.130	+ 2.96	+.023
0.882	0.128	− 0.92	−.008

Twenty-six analysts in sixteen laboratories analyzed natural water samples containing exact concentrations of orthophosphate, with the following results:

Known Concen. of Orthophosphate mg P/liter	Precision as Standard Deviation mg P/liter	Accuracy as Bias, %	Accuracy as Bias, mg P/liter
0.029	0.010	-4.95	-.001
0.038	0.008	-6.00	-.002
0.335	0.018	-2.75	-.009
0.383	0.023	-1.76	-.007

Calibration Requirements: Requires standard solutions

Data Outputs: Meter (analog voltage), manually recorded

Special Sampling Requirements Collection, Storage, Handling): If stored more than 8-10 hours, add mercuric chloride or preservative and refrigerate at 4°C.

References:

1. Methods for Chemical Analysis of Water and Wastes. 1971. EPA National Environmental Research Center, Cincinnati, Ohio.

PHOSPHORUS

Name of Measurement Method: Automated Single Reagent Method

Principal Detection Technique: Colorimetry

Purpose of Measurement (Important Applications): Ambient surface waters, domestic and industrial wastes, saline waters. (May also prove applicable to sediments, sludges.)

Summary of Method: Ammonium molybdate and potassium antimonyl tartrate react in an acid medium with dilute solutions of phosphorus to form an antimony-phosphomolybdate complex. This complex is reduced to an intensely blue-colored complex by ascorbic acid. The color is proportional ot the phosphorus concentration.

A Technicon AutoAnalyzer is used with a colorimeter equipped with 650 nm filter.

Limitations:

Range of Applicability: 0.01 to 1.0 mg/l phosphorus

Interferences: Iron in high concentrations; mercuric chloride, when used as a preservative, must be compensated for.

Pitfalls; Special Precautions: Benthic deposits must be avoided when sample is collected; glassware must not be cleaned with commercial detergents.

Statistical Characteristics; Six laboratories analyzed four natural water samples containing exact concentrations or orthophosphate, with the following results:

Known Conc. of Orthophosphate mg P/liter	Precision as Standard Deviation mg P/liter	Accuracy as Bias, %	Accuracy as Bias, mg P/liter
0.04	0.019	+16.7	+.007
0.04	0.014	- 8.3	-.003
0.29	0.087	-15.5	-.05
0.30	0.066	-12.8	-.04

Time of Measurement: About 20 samples per hour.

Calibration Requirement: Requires standard solutions, and standardized substitute ocean water when analyzing saline water. See Reference for calibration details.

Data Outputs: Analog electrical signal recorded on stripchart.

Special Sampling Requirement Collection, Storage, Handling): Avoid benthic deposits during collection. If not analyzed same day as collected, preserve with mercuric chloride and refrigerate at 4°C.

References:

1. Methods for Chemical Analysis of Water and Wastes. 1971. EPA National Environmental Research Center, Cincinnati, Ohio.

PHOSPHORUS

Name of Measurement Method: Automated Stannous Chloride Method

Principal Detection Technique: Colorimetry

Purpose of Measurement (Important Applications): Ambient surface waters, domestic and industrial wastes. (May prove applicable to sediment, sludge and algae blooms).

Summary of Method: Phosphorus is determined by manually digesting the samples with ammonium persulfate and sulfuric acid to convert the various forms of phosphorus to the orthophosphate form; and measurement of this orthophosphate on a Technicon AutoAnalyzer, using $(NH_4)_2MoO_4$ with $SnCl_2$ reduction to form color complex.

Limitations:

Range of Applicability: 0.01 to 1.0 mg/l phosphorus

Pitfalls; Special Precautions: Avoid benthic deposits when acquiring sample. Do not use commercial detergents for cleansing apparatus.

Statistical Characteristics: On a single laboratory, using surface water samples at concentrations of 0.06, 0.11, 0.48, and 0.62 mg P/liter, the standard deviation was ±0.004 (AQC Laboratory).

Time of Measurement: About 15 samples per hour

Calibration Requirements: Requires standard solutions. See Ref. (1) for details.

Data Outputs: Analog electrical signal recorded on stripchart.

Special Sampling Requirements Collection, Storage, Handling): Avoid benthic deposits when sampling. If sample is not analyzed on day of acquisition, preserve with mercuric chloride and refrigerate at 4~~½~~C.

References:

1. Methods for Chemical Analysis of Water and Wastes. 1971. EPA National Environmental Research Center, Cincinnati, Ohio.

SILICA

Principal Detection Tehcnique: Colorimetry

Purpose of Measurement (Important Applications): Drinking water, ambient surface waters, domestic and industrial wastes, saline waters.

Summary of Method: A well-mixed sample is filtered through a 0.45u membrane filter. The filtrate, upon the addition of molybdate ion in acidic solution, forms a greenish-yellow color complex proportional to the dissolved silica in the sample. The color complex is then measured spectrophotometrically.

Limitations:

Range of Applicability: 2 to 25 mg/l silica

Interferences: Excessive color or turbidity

Statistical Characteristics: Photometric evaluations by the amino-naphthol-sulfonic acid procedure have an estimated precision of $\underline{+}0.10$ mg/l in the range from 0 to 2 mg/l (ASTM). Photometric evaluation of the **silica**-molybdate color in the range from 2 to 50 mg/l have an estimated precision of approximately 4 percent of the quantity of silica measured (ASTM).

Data Outputs: Analog electrical signal

References:

1. Methods for Chemical Analysis of Water and Wastes. 1971. EPA National Environmental Research Center, Cincinnati, Ohio.

2. ASTM Standards. 1970. Part 23, Atmospheric Analysis Method D859-68.

OIL AND GREASE

Name of Measurement Method: Extraction/Gravimetric

Purpose of Measurement (Important Applications): Measures hexane-extractable matter (animal fats, non-volatile hydrocarbons, waxes, greases) in surface waters, industrial wastes, and domestic sewage.

Summary of Method: The sample is acidified to a low pH (4.3) and extracted with hexane using a Soxhlet extraction. The solvent is evaporated from the separated extract and the residue weighed.

Limitations:

Range of Applicability: 5 to 1000 mg/l of extractable matter

Statistical Characteristics: Precision and accuracy data not available from reference below.

Time of Measurement: Not stated, but several hours.

Data Outputs: Read from analytical balance.

References:

1. Methods for Chemical Analysis of Water and Wastes. 1971. EPA National Environmental Research Center, Cincinnati, Ohio.

pH

Name of Measurement Method: Electrometric

Principal Detection Technique: Electrometric

Purpose of Measurement (Important Applications): Drinking water, ambient surface waters, domestic and industrial waters, saline waters.

Summary of Method: The pH of a sample is an electrometric measurement, using either a glass electrode in combination with a reference potential (saturated calomel electrode) or a combination electrode (glass and reference).

Limitations:

Range of Applicability: Not stated, but assumed broad.

Interferences: Oil and grease.

Statistical Characteristics: Forty-four analysts in twenty laboratories analyzed six synthetic water samples containing exact concentrations of hydrogen-hydroxyl ions, with the following results:

Known Conc. as pH units	Precision as Standard Deviation pH units	Accuracy as Bias, %	Accuracy as Bias, pH units
3.5	0.10	-0.29	-0.01
3.5	0.11	-0.0	--
7.1	0.20	+1.01	+0.07
7.2	0.18	-0.03	-0.002
8.0	0.13	-0.12	-0.01
8.0	0.12	+0.16	+0.01

Time of Measurement: Not stated, but rapid (several per hour)

Calibration Requirements: Instrument must be initially standardized.

Comments by Users: Field pH measurements made with comparable instruments are reliable.

Data Outputs: Analog signal displayed on meter.

Special Sampling Requirements (Collection, Storage, Handling): Analyze as soon as possible after collection. No holding.

References:

1. Methods for Chemical Analysis of Water and Wastes. 1971. EPA National Environmental Research Center, Cincinnati, Ohio.

2. Standard Methods for Examination of Water and Wastewater. 1971. 13th Edition. American Public Health Association, Washington, D. C.

PHENOLICS

Name of Measurement Method: 4-AAP Method with Distillation

Principal Detection Technique: Colorimetry

Purpose of Measurement (Important Applications): Drinking water, ambient surface waters, domestic and industrial wastes, saline waters.

Summary of Method: Phenolic materials react with 4-aminoantipyrine in the presence of potassium ferricyanide at a pH of 10 to form a stable reddish-brown colored antipyrine dye. The amount of color produced is a function of the concentration of phenolic material.

Limitations:

Range of Applicability: 5 to 1000 g/l phenol (with solvent extraction)50 to 5000 g/l phenol (without solvent extraction)

Interferences: None stated. pH must be controlled

Pitfalls; Special Precautions: Method does not differentiate different types of phenolic materials.

Statistical Characteristics: The following results were obtained by analysts at six laboratories:

Known Concentration of Phenol (g/l)	Standard Deviation (ug/l) with Solvent Extraction
9.6	0.99
48.3	3.1
93.5	4.2

Calibration Requirements: Color response varies with type of phenolic material. Phenol is used as standard.

Data Outputs: Meter reading (analog electrical signal).

References:

1. Methods for Chemical Analysis of Water and Wastes. 1971. EPA National Environmental Research Center, Cincinnati, Ohio.

2. Standard Methods for Examination of Water and Wastewater. 1971. 13th Edition. American Public

Health Association, Washington, D. C.

SOLIDS
(DISSOLVED)

Principal Detection Technique: Filtration/Gravimetric

Purpose of Measurement (Important Applications): Ambient surface waters, domestic and industrial wastes, saline waters.

Summary of Method: A well mixed sample is filtered through a standard glass fiber filter. The filtrate is evaporated and dried to constant weight at 180°C.

Limitations:

Range of Applicability: 10 to 20,000 mg/l solids

Pitfalls; Special Precautions: Mineralized waters containing calcium, magnesium, chloride and/or sulfate may be hygroscopic and will require prolonged drying and quick weighing. Samples containing bicarbonates require careful drying at 180°C to insure conversion to the carbonate.

Statistical Characteristics: Precision data not available at this time. References below. Accuracy data on actual sample cannot be obtained.

Data Outputs: Read from analytical balance.

Special Sampling Requirements (Collection, Storage, Handling): Sample should be analyzed as soon as possible after acquisition.

References:

1. Methods for Chemical Analysis of Water and Wastes. 1971. EPA National Environmental Research Center, Cincinnati, Ohio.

SOLIDS
(SUSPENDED)

Principal Detection Technique: Filtration/Gravimetric

Purpose of Measurement (Important Applications): Ambient surface waters, domestic and industrial wastes, saline waters.

Summary of Method: A well-mixed sample is filtered through a standard glass fiber filter, and the residue retained on the filter is dried to constant weight at 103-105 °C. Non-filterable solids are defined as those solids which are retained by a standard glass fiber filter and dried to constant weight at 103-105°C.

Limitations:

Range of Applicability: 20 to 20,000 mg/1 solids

Pitfalls; Special Precautions: Too much residue on the filter will entrap water and require polonged drying.

Statistical Characteristics: Reproduction of the data not available at this time. Accuracy data on actual samples cannot be obtained.

Data Outputs: Analytical balance reading.

Special Sampling Requirements (Collection, Storage, Handling): Preservation of sample is not practical; analysis should begin as soon as practical. Non-homogeneous particulates (sticks, fish, etc.) should be excluded from sample.

References:

1. Methods for Chemical Analysis of Water and Wastes. 1971. EPA National Environmental Research Center, Cincinnati, Ohio.

SOLIDS
(TOTAL)

Principal Detection Technique: Filtration/Gravimetric

Purpose of Measurement (Important Applications): Ambient surface waters, domestic and industrial wastes, saline waters.

Summary of Method: A well mixed aliquot of the test sample is quantitatively transferred to a pre-weighed evaporating dish and evaporated to dryness at 103-105°C. (Total Solids are defined as the sum of the homogeneous suspended and dissolved materials in a sample).

Limitations:

Range of Applicability: 10 to 30,000 mg/l solids

Pitfalls; Special Precautions: Floating oils and greases, if present, should be dispersed with a blender. Large floating particles should be excluded.

Statistical Characteristics: Precision and accuracy data not available at this time.

Data Outputs: Read from analytical balance

References:

1. Methods for Chemical Analysis of Water and Wastes. 1971. EPA National Environmental Research Center, Cincinnati, Ohio.

SOLIDS
(VOLATILE)

Principal Detection Technique: Ignition/Filtration

Purpose of Measurement (Important Applications): The test is useful in obtaining a rough approximation of the amount of organic matter present in the solid fraction of sewage, activated sludge, industrial wastes, or bottom sediments.

Summary of Method: The residue obtained from the determination of total, suspended, or dissolved solids is ignited at 550°C in a muffle furnace. The loss of weight on ignition is reported as mg/l volatile solids.

Limitations:

Pitfalls; Special Precautions: The test is subject to many errors due to loss of water of crystallization, loss of volatile organic matter prior to combustion, incomplete oxidation of certain complex organics, and decomposition of mineral salts during combustion.

Statistical Characteristics:

Reproducibility: A collaborative study involving three laboratories examining four samples by means of ten replicates showed a standard deviation of ±11 mg/l at 170 mg/l volatile solids concentration.

Data Outputs: Read from analytical balance.

References:

1. Methods for Chemical Analysis of Water and Wastes. 1971. EPA National Environmental Research Center, Cincinnati, Ohio.

2. Standard Methods for Examination of Water and Wastewater. 1971. 13th Edition, American Public Health Association, Washington, D. C.

SULFATE

Principal Detection Technique: Colorimetry

Purpose of Measurement (Important Applications): Drinking water, ambient surface waters, domestic and industrial wastes.

Summary of Method: Sulfate ion is converted to a barium sulfate suspension under controlled conditions. The resulting turbidity is determined by a photoelectric colorimeter or spectophotometer and compared to a curve prepared from standard sulfate solutions.

Limitations:

Range of Applicability: All concentrations of sulfate ion. Higher concentrations must be diluted such that sample aliquot contain less than 40 mg/l sulfate.

Interferences: Suspended matter and color interfere. Correct by running blanks from which the barium chloride has been omitted.

Statistical Characteristics: Thirty-four analysts in 16 laboratories analyzed six synthetic water samples containing exact concentrations of inorganic sulfate with the following results:

Known Conc. of Sulfate mg/liter	Precision as Standard Deviation mg/liter	Accuracy as Bias %	Accuracy as Bias mg/liter
8.6	2.30	-3.72	-.3
9.2	1.78	-8.26	-.8
110.0	7.86	-3.01	-3.3
122.0	7.50	-3.37	-4.1
188.0	9.58	+0.04	+.1
199.0	11.8	-1.70	-3.4

Time of Measurement: Rapid

Data Outputs: Meter output (analog electrical signal), manually

References:

1. Methods for Chemical Analysis of Water and Wastes. 1971. EPA National Environmental Research Center, Cincinnati, Ohio.

2. Standard Methods for Examination of Water and Wastewater. 1971. 13th Edition. American Public Health Association, Washington, D. C.

SULFATE

Name of Measurement Method: Automated Chloranilate Method

Principal Detection Technique: Colorimetry

Purpose of Measurement (Important Applications): Ambient surface waters, domestic and industrial wastes, saline waters.

Summary of Method: When solid barium chloranilate is added to a solution containing sulfate, barium sulfate is precipitated, releasing the highly colored acid chloranilate ion. The color intensity in the resulting chloranilic acid is proportionate to the amount of sulfate present.

Method employs Technicon AutoAnalyzer with colorimeter equipped with 520 nm filters.

Limitations:

Range of Applicability: 10 to 400 mg/l sulfate

Interferences: Calcium, aluminum, iron interfere, but may be removed by ion exchange.

Statistical Characteristics: In a single laboratory (AQC), using surface water samples at concentrations of 39, 111, 188, and 194 mg SO_4/l, the standard deviations were ±0.6, ±1.0, ±2.2, and ±0.8, respectively.

Time of Measurement: About 15 samples per hour.

Calibration Requirements: Requires preparation of standard solutions and calibration curve.

Data Output: Analog electrical signal, recorded on stripchart.

Special Sampling Requirements (Collection, Storage, Handling): Refrigerate at 4 C.

References:

1. Methods for Chemical Analysis of Water and Wastes. 1971. EPA National Environmental Research Center, Cincinnati, Ohio.

SULFIDE

Name of Measurement Method: Titrimetric (Iodine) Method

Principal Detection Technique: Titration

Purpose of Measurement (Important Applications): Drinking waters, ambient surface waters, domestic and industrial wastes, saline waters.

Summary of Method: Sulfides are stripped from the acidified sample with an inert gas and collected in a zinc acetate solution. Excess iodine added to the zinc sulfide suspension reacts with the sulfide under acidic conditions. Thiosulfate is used to measure unreacted iodine to indicate the quantity of iodine consumed by sulfide.

Limitations:

Range of Applicability: Concentrations of sulfide above one mg/l

Interferences: Sulfites, thiosulfates, hydrosulfates, and other reduced sulfur compounds may interfere.

Pitfalls; Special Precautions: Sample should have minimal contact with air or oxygen.

Statistical Characteristics: Precision and accuracy for this method have not been determined, but it is claimed that the iodimetric titration of the zinc sulfide is quite accurate.

Data Outputs: Not stated. Assumed to be visual observations manually recorded.

Special Sampling Requirements (Collection, Storage, Handling): Minimize contact with air. Preserve with zinc acetate unless analysis is performed immediately.

References:

1. Methods for Chemical Analysis of Water and Wastes. 1971. EPA National Environmental Research Center, Cincinnati, Ohio.

2. Standard Methods for Examination of Water and Wastewater. 1971. 13th Edition. American Public Health Association, Washington, D. C.

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TURBIDITY

Name of Measurement Method: Nephelometric

Principal Detection Technique: Photometric (Nephelometer)

Purpose of Measurement (Important Applications): This method is applicable to surface and saline waters in the range of turbidity from 0 to 40 Jackson units.

Summary of Method: The method is based upon a comparison of the intensity of light scattered by the sample under definite conditions with the intensity of light scattered by a standard reference suspension. The higher the intensity of scattered light, the higher the turbidity. Readings, in Jackson units, are made in a nephelometer designed according to specifications outlined in Reference cited below. A standard suspension of Formazin, also prepared under closely defined conditions, is used as the turbidity reference suspension for water because it is more reproducible than other types of standards previously used for turbidity standards. Design criteria for instruments are given in Reference.

Limitations: Range of Applicability: 0 to 40 Jackson units. Sensitivity should be 0.02 units or less in waters having turbidity of less than one unit.

Interferences: Floating debris, coarse sediments, air bubbles, or colored material on solution may interfere.

Pitfalls; Special Precautions: Care should be exercised in selection of instrument that meets criteria given in Reference.

Statistical Characteristics:

Accuracy and Precision: Data not available at time of publication of Reference.

Time of Measurement: Not stated. Assumed rapid but not instantaneous, because sufficient time must be allowed after shaking sample for air bubbles to disappear but not for all suspended particles to settle. (Five minutes or less).

Calibration Requirements: Follow instrument manufacturer's recommendations, but reliance on manufacturer's solid scattering standard is not always acceptable. See Reference below.

Data Outputs: Photoelectric detector (analog signal) with ammeter readout.

Special Sampling Requirements (Collection, Storage, Handling):

Samples taken for turbidity measurements should be analyzed as soon as possible. Preservation of samples is not recommended.

References:

1. Methods for Chemical Analysis of Water and Wastes. 1971. EPA National Environmental Research Center, Cincinnati, Ohio.

TEMPERATURE

Principal Detection Technique: Thermometry

Purpose of Measurement (Important Applications): Drinking waters, ambient surface waters, domestic and industrial wastes, saline waters.

Summary of Method: Temperature measurements may be made with any good grade of mercury-filled or dial-type centigrade thermometer, or a thermistor.

Limitations:

Range of Applicability: Thermometers or thermistors can be obtained to give valid results over almost any range of ambient temperatures.

Pitfalls; Special Precautions: Measurement device should be checked against a precision thermometer certified by the National Bureau of Standards.

Statistical Characteristics: There is no acceptable procedure for determining the precision and accuracy of this test.

Time of Measurement: Rapid

Data Outputs: Usually a visual reading manually recorded. Instruments producing analog voltages automatically recorded are readily available.

References:

1. Methods for Chemical Analysis of Water and Wastes. 1971. EPA National Environmental Research Center, Cincinnati, Ohio.

2. Standard Methods for Examination of Water and Wastewater. 1971. 13th Edition. American Public Health Association, Washington, D.C.

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THRESHOLD ODOR

Name of Measurement Method: Consistent Series Method

Principal Detection Technique: Personal Sensing

Purpose of Measurement (Important Applications): This method is applicable to the determination of threshold odor or finished waters, surface waters, domestic and industrial wastes, and saline waters.

Summary of Method: The sample of water is diluted with odor-free water until a dilution that is of the least definitely perceptible odor to each tester is found. The resulting ratio by which the sample has been diluted is called the "threshold odor number" (T.O.).

People vary widely as to odor sensitivity, and even the same person will not be consistent in the concentrations he can detect from day to day. Therefore, panels of not less then five persons, and preferably 10 or more, are recommended to overcome the variability of using one observer.

As an absolute minimum, two persons are necessary: one to make the sample dilutions and one to determine the threshold odor.

Limitations:

Range of Applicability: Highly odorous samples are reduced in concentration proportionately before being tested. Thus, the method is applicable to samples ranging from nearly odorless natural waters to industrial wastes with threshold odor numbers in the thousands.

Interferences: Chlorine in tap water and some wastewaters may affect results. It is sometimes desirable to determine the odor of the chlorinated sample and the same sample after removal of the chlorine. Removal can be accomplished by use of sodium thiosulfate in stoichiometric proportions.

Pitfalls; Special Precautions: It is important to check a blank to which a similar amount of a dechlorinating agent has been added to determine if any odor has been imparted. Such odor usually disappears upon standing if excess reagent has not been added.

Statistical Characteristics:

Accuracy and Precision: Data not available at the time of publication of Reference cited below.

Time of Measurements: Several hours.

Calibration Requirements: See Reference

Data Outputs: Personal detection of odor

Special Sampling Requirements (Collection, Storage, Handling): Water samples must be collected in glass bottles with glass or Teflon-lined closures. Plastic containers are not reliable for odor samples and must not be used. Complete test as soon as possible after sample collection. If storage is necessary, fill container to the top and refrigerate.

References:

1. Methods for Chemical Analysis of Water and Wastes. 1971. EPA National Environmental Research Center, Cincinnati, Ohio.

SPECIFIC CONDUCTANCE

Principal Detection Technique: Electrometric

Purpose of Measurement (Important Applications): Drinking water, ambient surface waters, domestic and industrial wastes, saline waters.

Summary of Method: The specific conductance of a sample is measured by use of a self-contained conductivity meter, Wheatstone bridge-type, or equivalent. Samples are preferably analyzed at 25°C. If not, temperature corrections are made and results reported at 25°C.

Statistical Characteristics: Forty-one analysts in 17 laboratories analyzed six synthetic water samples containing known concentrations of inorganic salts, with known values of specific conductance. The following results were obtained:

Known Conc. of Specific Conductance mhos/cm	Precision as Standard Deviation mhos/cm	Accuracy as Bias %	Bias, mhos/cm
100	7.55	-2.02	-2.0
106	8.14	-0.76	-0.8
808	66.1	-3.63	-29.3
848	79.6	-4.54	-38.5
1640	106	-5.36	-87.9
1710	119	-5.08	-86.9

In a single laboratory (AQC), using surface water samples with an average conductivity of 536 mhos/cm at 25°C, the standard deviation was ±6.

Calibration Requirements: Meter reading (analog electrical signal, manually recorded).

References:

1. Methods for Chemical Analysis of Water and Wastes. 1971. EPA National Environmental Research Center, Cincinnati, Ohio.

2. Standard Methods for Examination of Water and Wastewater. 1971. 13th Edition. American Public Health Association, Washington, D.C.

IDENTIFICATION OF WEATHERED OR UNWEATHERED OILS

Medium: Water, sediments, and tissue

Name of Measurement Method: Woods Hole Oceanographic Institute Method

Principal Detection Techniques: Gas chromatography (flame ionization)

Purpose of Measurement (Important Applications): Passive tagging of oils

Summary of Method: Oils are dissolved in carbon disulfide and injected into the gas chromatography GS. The column is 50 feet x 0.02 inches (open tubular support coated--SCOT) column packed with nonpolar liquid silicone OV-101, rated at 25,000 effective plates. Oil chromatograms are compared visually, and certain features are abstracted, tabulated and compared to the chromatograms generated by candidate unweathered oil samples.

Limitations:

Interferences: Samples which have undergone unusual bacterial alteration contain high levels of indigenous hydrocarbons; mixtures of oils and samples having under gone unusually prolonged weathering require special treatment.

Pitfalls; Special Precautions: Tabulated indices of oils that exhibit considerable weathering should not be compared directly with the indices of unweathered oils.

Statistical Chracteristics:

Accuracy: 16 out of 17 unweathered oils used for this test accompanied 35 simulated oil spill samples. Correct "definite correlation" was achieved in 74 percent of the cases, and only one "probable correlation" was incorrect.

Precision: Tabulated indices of individual oils remain quite stable from column to column.

Calibration Requirements: Method requires frequent evaluation of system performance by injecting a standard oil that yields a repeatable known chromatogram.

Data Outputs: Analog instrument reading, graphically recorded.

Special Sampling Requirements (Collection, Storage, Handling):

Sample should be refrigerated if held for more than a few days. Glass or metal containers are required for sampling.

References:

1. Zaifirion, O. M. Blumer and J. Myers. 1972. Correlation of Oils and Oil Products by Gas Chromatography. Woods Hole Oceanographic Institution Report 62-55.

2. Kawahara, Fred K.b 1969. Laboratory Guide for the Identification of Petroleum Products, Federal Water Pollution Control Administration, Division of Water Quality Research, Cincinnati, Ohio.

PETROLEUM OILS

Name of Measurement Method: Quantitative Analysis of Oil in Water Dispersion

Principal Detection Techniques: Infrared Spectrophotometry

Purpose of Measurement (Important Applications): Identification of the particular oil and its quantitative determinations in the water column are essential to properly monitor and assess potential biological damage resulting from oil spill incidents.

Summary of Method: Salt and acid added to the sample which is then extracted with carbon tetrachloride or Freon 113, using a separatory funnel. The extract is measured by infrared spectrophotometry.

Limitations:

Sensitivity: Minimum detection limit 0.05 mg/l

Interferences: (Freon or CCl_4) - Any solvent extractable organics.

Pitfalls; Special Precautions: Carbon tetrachloride has a TLV of 10 mg/l.

Statistical Characteristics:

Time of Measurement:

Calibration Requirements: Freon is not usable for preparing IR standards of heavy oils (2).

Comments by Users: Carbon tetrachloride is more efficient than Freon 113 for extracting high concentrations of viscous oils (1000 centistokes at 100 F) from water dispersion.

Data Outputs: Analog instrumental reading.

Special Sampling Requirements (Collection, Storage, Handling): Sample is collected in a glass-stoppered bottle and acidified at the time of collection.

References:

1. Kawahara, Fred K. 1969. Laboratory Guide for the Identification of Petroleum Products, Federal Water Pollution Control Administration, Division of Water Quality Research, Cincinnati, Ohio.

PESTICIDE

Principal Detection Techniques: Electron Capture, Gas Liquid Chromatography

Purpose of Measurement (Important Applications): To determine presence and identification of pesticides in waters.

Summary of Method: The water sample is subject to multiple extraction by ethyl ether/hexane. The combined extracts are dried with anhydrous sodium sulfate, evaporated under a stream of nitrogen, and further concentrated by heating. Aliquots of about 5 ul are used for the initial electron capture gas liquid chromatography. Further identification may be necessary through microcoulometry of thin layer chromatography.

Limitations:

Interferences: If interferences are indicated in initial chromatograms, it may be necessary to conduct a Florisil clean up on the extract.

Calibration Requirements: See Reference

Data Outputs: Analog electrical signal displayed as chromatogram.

Special Sampling Requirements (Collection, Storage, Handling): Sampling collection glassware should be scrupulously cleaned. If storage is necessary samples should be kept in cool, dark place or preferably in a refrigerator.

Reference:

1. Thompson, J. F. (Editor). 1971. Analysis of Pesticide Residues in Human and Environmental Samples. EPA Primate and Pesticides Effects Laboratory, Perrine, Florida.

PESTICIDES

Parameter(s) Measured: Organochlorine Pesticides

Aldrin
BCH
Chlordane
DDD
DDE
DDT
Dieldrin
Endosulfan
Endrin
Heptachlor
Heptachlor Epoxide
Chlorodane (tech)
Methexychlor
Perthane
Sulphenone

under some circumstances:

Kelthane
Strobane
Toxaphene

Principal Detection Techniques: Electron Capture (EC)
Microcoulometric Titration (MC)
Electrolytic Conductivity (ECD)

Purpose of Measurement (Important Applications): For identification and quantitation of various organochlorine pesticides, certain degradations products, and related compounds.

Summary of Method: The method offers several analytical alternatives, depending on the complexity of pesticides in the sample and the nature and amounts of interferences. In general, the pesticides in the aqueous sample are extracted by organic solvents. (e.g., hexane or hexane/ethyl ether mixtures), and the extract concentrated by careful evaporation of the solvent. Removal of interferences (when necessary) and pre-separation of pesticide mixtures are accomplished by column chromatography, thin-layer chromatography, or liquid-liquid partitioning. Identification of pesticides in the mixture is made by selective gas chromatographic separations through the use of two or more unlike columns. Detection and measurement of pesticides in the sample are accomplished by electron capture, microcoulometric, or electrolytic conductivity techniques. Quantitative results are obtained by measurement of areas under peaks in the resulting chromatogram.

The reference cited describes the use of an effective solvent for extraction of pesticides from aqueous samples. It provides information on the selection of appropriate clean-up procedures and detectors for various types of pesticide mixtures, and suggests techniques for confirming qualitative identifications.

Limitations:

Range of Applicability: Specific concentration ranges for various pesticides in environmental samples are not cited, but the detection capabilities of four types of detectors are indicated below.

Sensitivity and Detection Limits:

Electron Capture Detector picogram (110 gram) quantities of many organochlorine pesticides

Microcoulometric Titration 5-20 ng of organochlorine pesticides

Electrolytic Conductivity Sensitivity Detector 2 to 3 times as great as microcoulometric procedure

Flame Photometric Detector Sub-nanogram quantities of sulfur and phosphorus

Sample responses less then two times the detector noise level (N) should be reported as negative; responses greater than 2N should be quantified if possible.

Interferences: Polychlorinated biphenyls; phthalate esters, organophosphorus pesticides. The presence of the latter two classes of compounds are implicated in samples that respond to certain detection techniques but not to others.

Pitfalls; Special Precautions: Contaminants in solvents, reagents, glassware, and other equipment may yield results that cause misinterpretation of chromatograms.

Statistical Characteristics:

Accuracy: Not stated as percent relative error, but given as percent recovery in Ref.

Precision: Results are given for four specific pesticides. Those for aldrin are as follows:

	Mean recovery (ng/liter)	Precision (ng/liter)	
		Overall	Single Operation
No cleanup	10.42	4.86	2.59
	79.00	32.06	20.19
Florisil cleanup	17.00	9.13	3.48
	64.54	27.16	8.02

Calibration Requirements: Procedures are detailed in Reference.

Comments by Users: Recommended for use only by, or under close supervision of, experienced residue analysts.

Data Outputs: Analog electrical signals recorded on stripcharts.

Special Sampling Requirements (Collection, Storage, Handling): Wide-mouth bottles with Teflon-lined screw caps are used for sample collection.

References:

1. Methods for Organic Pesticides in Water and Wastewater. 1971.
EPA National Environmental Research Center, Cincinnati, Ohio.

2. BIOLOGICAL METHODS

PHYTOPLANKTON AND PERIPHYTON
CELL COUNTS AND IDENTIFICATION

Principal Detection Technique: Visual Observation

Purpose of Measurement (Important Applications): Provide information on the standing crops, indicator organisms and community diversity.

Summary of Method: A 24x60 mm, No. 1 coverglass is placed diagonally across the S-R cell and a large bore pipet or eyedropper is used to transfer a one/ml aliquot of a well-mixed sample into the open corner of the Sedgwick-Rafter chamber. The S-R cell is allowed to stand for at least 15 minutes to permit settling. The analysis may then proceed in either of two ways: (1) depending on the density of organisms, two to four "strips" the length of the cells are examined and all forms that are totally or partially covered by the whipple-grid are enumerated; or (2) a minimum of ten random whipple fields are examined in at least two identically prepared S-R cells and forms that are totally or partially covered by the whipple grid are enumerated.

Limitations:

Range of Applicability: Phytoplankton and periphyton

Interferences: In samples where algae concentrations are extreme or where turbidity is high the sample must be diluted or concentrated.

Pitfalls; Special Precautions: The depth of the counting chamber precludes the use of the 45x or 100x objectives. Collection of phtoplankton by nets or pumps is not recommended.

Statistical Characteristics:

Precision: Provided reasonably reproducible information when used with a calibrated microscope with an eyepiece measuring device.

Time of Measurement: 1 hour

Calibration Requirements: Each combination of oculars and objectives is calibrated against a stage micrometer.

Data Outputs: Manual count

Special Sampling Requirements (Collection, Storage, Handling): The pretreatment of the sample depends on the concentration of organisms present. When phytoplankton densities are less then 500/ml, approximately 6 liters of sample are required.

References:

1. Biological Field and Laboratory Methods. 1972. EPA National Environmental Research Center, Analytical Quality Control Laboratory, Cincinnati, Ohio. Preliminary Draft.

VOLUME OF PERIPHYTON

Principal Detection Technique: Volumetric

Purpose of Measurement (Important Applications): Estimate of the standing stock of periphyton.

Summary of Method: A known volume of water is added to a thoroughly drained sample. The difference between the volume of the periphyton samples plus the added water and the volume of water alone is the volume of the total amount of periphyton in the sample.

Limitations:

Range of Applicability: Periphyton, only when large growths of periphyton permit removal of excess water readily.

Pitfalls; Special Precautions: Excess fluid should be removed from the sample.

Calibration Requirements: Graduated cylinder should be used for volume measurements.

Data Outputs: Volume measurement (visual)

Special Sampling Requirements (Collection, Storage, Handling): The preferred preservative is neutral or slightly basic formalin.

References:

1. Biological Field and Laboratory Method. 1972. EPA National Environmental Research Center, Analytical Quality Control Laboratory, Cincinnati, Ohio. Preliminary Draft.

CELL COUNTS AND IDENTIFICATION OF PHYTOPLANKTON

Principal Detection Techniques: Visual observation

Purpose of Measurement (Important Applications): Provides information on standing crop, indicator organisms and community diversity.

Summary of Method: The sample is introduced into cells which are precisely matched glass slides with a finely-ruled grid on a counting plate fitted with a specially ground cover slip. All forms which fall within the gridded area of the cell are identified and counted. The number of the various organisms found in the gridded area of the cell is multiplied by the appropriate factor to obtain the count.

Limitations:

Pitfalls; Special Precautions: The analyst is advised to follow carefully the specific directions accompanying the chamber or cell. Collection of phytoplankton by nets or pumps is not recommended. For statistical purposes a normal sample must be either concentrated or a large number of mounts per sample should be examined.

Statistical Characteristics:

Time or Measurement: 1 hour

Data Outputs: Manual count

Special Sampling Requirements (Collection, Storage, Handling: Depends upon the location and type of sample. See Reference. Samples must be concentrated when densities are below 500/ml. Approximately 6 liters of sample are required.

References:

1. Biological Field and Laboratory Methods. 1972. EPA National Environmental Research Center, Analytical Quality Control Laboratory, Cincinnati, Ohio. (Preliminary Draft).

CELL COUNTS AND IDENTIFICATION OF PHYTOPLANKTON

Principal Detection Technique: Visual observation (Filter Method)

Purpose of Measurement (Important Applications): Provides information on standing crop, indicator organisms and community diversity. Method permits the use of high magnification for enumeration of small plankton.

Summary of Method: A water sample of known volume is passed through the membrane filter under a vacuum. The filter is allowed to clear and the organisms enumerated. The occurrence of each species in 30 random fields is recorded and multiplied by a conversion factor to obtain the total count of each species.

Interferences: Significant amounts of suspended matter may obscure or crush the organisms.

Pitfalls; Special Precautions: In coastal and marine waters the filter is rinsed with distilled water to remove salt. Collection of phytoplankton by nets or pumps is not recommended.

Statistical Characteristics:

Precision: ± 16% CL

Time of Measurement: Relatively rapid processing of samples, (1 hour) although total time required for the complete analysis is 48-50 hours.

Calibration Requirements: Use stage and ocular micrometers

Data Outputs: Manual count

Special Sampling Requirements (Collection, Storage, Handling: Depends upon the location and type of sample taken. See Reference. Samples must be concentrated when phytoplankton densities are less than 500/ml. Approximately 6 liters of sample are required.

References:

1. Biological Field and Laboratory Methods. 1972. EPA National Environmental Research Center, Analytical Quality Control Laboratory, Cincinnati, Ohio. (Preliminary Draft).

CELL COUNTS AND IDENTIFICATION OF PHYTOPLANKTON

Principal Detection Technique: Visual observation (Counting Chamber Method)

Purpose of Measurement (Important Applications): Provides information on algal standing crop, indicator organisms and community diversity.

Summary of Method: The method uses an inverted microscope in conjunction with a cylindrical counting chamber with a clear glass bottom. A sample is transferred to the counting chamber and allowed to settle. The chamber is placed on the microscope stage and examined using either the 20x, 45x or 100x oil immersion lens. Either the strip or random field counts are made.

Limitations:

Interferences: None identified

Pitfalls; Special Precautions: Collection of phytoplankton by nets or pumps is not recommended.

Statistical Characteristics:

Time of Measurement: 4 hours per 10 mm of sample height in the chamber is allowed for sedimentation. Count requires 1 hour.

Calibration Requirements: Use stage and ocular micrometers

Data Outputs: Manual count

Special Sampling Requirements (Collection, Storage, Handling): Depends upon the location and type or sample taken. See Reference.

References:

1. Biological Field and Laboratory Methods. 1972. EPA National Environmental Research Center, Analytical Quality Control Laboratory, Cincinnati, Ohio. (Preliminary Draft).

CELL VOLUME ESTIMATES OF PLANKTON AND PERIPHYTON

Principal Detection Technique: Visual

Purpose of Measurement (Important Applications): Standing crop estimate gives an indication as to water productivity.

Summary of Method: An aliquot of sample is concentrated and examined wet at a 1000 x magnification with a microscope equipped with a calibrated ocular micrometer. The optical measurements are made with the micrometer. The average volume per organism is determined and multiplied by the number of organisms per milliliter.

Limitations:

Range of Applicability: Phytoplankton, bacteria, periphyton

Calibration Requirements: The exact magnification with any set of oculars must be calibrated.

Data Outputs: Visual examination

Special Sampling Requirement (Collection, Storage, Handling): The preferred preservative is neutral or slightly basic formalin.

Reference:

1. Biological Field and Laboratory Methods. 1972. EPA National Environmental Research Center, Analytical Quality Control Laboratory, Cincinnati, Ohio. (Preliminary Draft).

PERIPHYTON AND PHYTOPLANKTON
(Species Composition)

Principal Detection Technique: Visual examination

Purpose of Measurement (Important Application): The identification of individual species of phytoplankton may provide information on indicator organisms, species diversity, and therefore the degree of pollution.

Summary of Method: Following an initial examination of the sample to obtain an estimate of population etc., the phytoplankters are identified to the desired taxonomic level and tallied under a standard system.

Limitations:

Range of Applicability: Plankton, periphyton

Pitfalls; Special Precautions: Examination is preferably done before sample is preserved. The beginner is strongly warned against the deceiving and nonvalid simplicity often found in the identification of plankton.

Statistical Characteristics:

Time of Measurement: 1 hour

Data Outputs: Numbers of species and the number of individuals per species

Special Sampling Requirement (Collection, Storage, Handling): In cases where it has been shown that preservation has no effects on the identification of organisms, a preservative should be used if storage is necessary.

References:

1. Biological Field and Laboratory Methods. 1972. EPA National Environmental Research Center, Analytical Quality Control Laboratory, Cincinnati, Ohio. (Preliminary Draft).

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DIATOM SPECIES IDENTIFICATION

Principal Detection Technique: Visual Observation

Purpose of Measurement (Important Applications): Provides information on indicator organisms and community diversity.

Summary of Method: The diatoms are concentrated by centrifugation followed by sedimentation. The diatom concentrate is placed on an 18 mm coverglass and dried on a hotplate at 95°C followed by the oxidization of organic matter. The hot coverglass is inverted and placed on a drop of hyrax on a 25x75 mm microscope slide. A protective coating of clear lacquer is sprayed on the frosted end of the slide and the excess hyrax is scraped from around the coverglass. Two hundred fifty diatoms are identified and counted at high magnification under oil. If the slide has very few diatoms the analysis is limited to the number of cells encounted in 45 minutes of scanning.

Limitations:

Range of Applicability: Diatoms

Interferences: Silt

Pitfalls; Special Precautions: If the dried sample is obscured by soluble solids, the sample should be washed with distilled water.

Statistical Characteristics:

Time of Measurements: 1 hour

Calibration Requirements: Ocular and stage micrometer

Data Outputs: Number of individuals per species

Special Sampling Requirements (Collections, Storage, Handling): Depends upon the location and type of sample taken, see Reference. If plankton counts are less than 1000 per ml, the diatoms should be concentrated from a larger volume of sample (one to five liters) by allowing them to settle out.

References:

1. Biological Field and Laboratory Methods. 1972. EPA National Environmental Research Center, Analytical Quality Control Laboratory, Cincinnati, Ohio. (Preliminary Draft).

CELL COUNTS AND IDENTIFICATION OF PHYTOPLANKTON

Principal Detection Techniques: Visual observation

Purpose of Measurement (Important Applications): Provides information on standing crop indicator organisms and community diversity.

Summary of Method: An aliquot of well mixed sample is introduced into one of the 2x5 mm channels on either side of the circular Palmer-Maloney Nannoplankton cell with cover slip in place. After 10 minutes, the sample is examined under the high-dry objective (45x) and at least 20 whipple fields are counted.

Limitations:

Range of Applicability: The circular chamber used was especially designed for enumerating nannoplankton with a high-dry objective (45x).

Pitfalls; Special Precautions: The cell should not be used for routine counting unless the samples have counts exceeding 20,000/ml. Collection of phytoplankton by net or pumps is not recommended.

Statistical Characteristics:

Time of Measurement: 1 hour

Calibration Requirements: The microscope is calibrated using an ocular and stage micrometer.

Comments by Users:

Data Outputs: Manual counts

Special Sampling Requirement (Collection, Storage, Handling): Depends upon the location and type of sample. See Reference. Samples must be concentrated when densities are below 500/ml. Approximately 6 liters of sample are required.

References:

1. Biological Field and Laboratory Methods. 1972. EPA National Environmental Research Center, Analytical Quality Control Laboratory, Cincinnati, Ohio. (Preliminary Draft).

CHLOROPHYLL a OF PHYTOPLANKTON
(In vitro)

Name of Measurement Method: Monochromatic Method

Principal Detection Technique: Spectrophotometric

Purpose of Measurement (Important Applications): All algae contain chlorophyll a. Measurement of this pigment can yield an estimate of standing crop.

Summary of Method: Chlorophyll a can be estimated independently of the other chlorophylls by measuring the optical density of the pigment extract of 665 nm only, and inserting it into the following equation: $C_a = 13.4D665$. (D665 is the optical density, corrected for turbidity by substracting the OD_{750} nm blank).

Limitations:

Pitfalls; Special Precautions: Precautions should be taken to minimize evaporation. Pheophytin a natural degradation product of chlorophyll, has an absorption peak in the same region of the visible spectrum as chlorophyll a and could be a source of error in chlorophyll determinations.

Calibration Requirements: No unusual requirements

Data Outputs: Analog or digital electrical signal displayed on meter, on tape or recorder.

Special Sampling Requirements (Collection, Storage, Handling): The type of sampling equipment used is highly dependent upon where and how the sample is being taken. If the analysis will be delayed, store the sample frozen in the dark to avoid photochemical breakdown of the chlorophyll.

References:

1. Biological Field and Laboratory Methods. 1972. EPA National Environmental Research Center, Analytical Quality Control Laboratory, Cincinnati, Ohio. (Preliminary Draft).

CHLOROPHYLL *a* OF PHYTOPLANKTON

Principal Detection Technique: Spectrophotometric

Purpose of Measurement (Important Applications): All algae contain chlorophyll *a*. Measurement of this pigment can yield an estimate of standing crop.

Summary of Method: Chlorophyll *a* can be estimated independently of the other chlorophylls by measuring the optical density of the pigment extract of 665 nm only, and inserting it into the following equation: $C_a = 13.4D_{665}$ (D_{665} is the optical density, corrected for turbidity by subtracting the OD 750 nm blank).

Limitations:

Pitfalls; Special Precautions: Precautions should be taken to minimize evaporation. Pheophytin a natural degradation product of chlorophyll, has an absorption peak in the same region of the visible spectrum as chlorophyll *a* and could be a source of error in chlorophyll determinations.

Calibration Requirements: No unusual requirements.

Data Outputs: Analog or digital electrical signal displayed on meter, on tape or recorder.

Special Sampling Requirements (Collection, Storage, Handling): The type of sampling equipment used is highly dependent upon where and how the sample is being taken. If the analysis will be delayed, store the sample frozen in the dark to avoid photochemical breakdown of the chlorophyll.

References:

1. Biological Field and Laboratory Methods. 1972. EPA National Environmental Research Center, Analytical Quality Control Laboratory, Cincinnati, Ohio. (Preliminary Draft).

ZOOPLANKTON VOLUME AND SPECIES IDENTIFICATION

Purpose of Measurement (Important Applications) Measurement of zooplankton. Volume provides an index to the standing crop (biomass) of natural zooplankton population.

Summary of Method: The sample is screened, placed into a conical container graduated in milliliters, allowed to settle for five minutes, and the settled volume is recorded. The sample is then stirred and a one ml subsample is withdrawn from the container with a Stempel pipette. The subsample is examined under a dissecting microscope for enumeration of zooplankton.

Limitations:

Range of Applicability: Rotifera, cladocera, copepods and other large zooplankton forms.

Statistical Characteristics:

Time of Measurement: Less than 1 hour

Data Outputs: Visual observation, manual reading

Special Sampling Requirements (Collection, Storage, Handling): Depends upon location and type of sample taken. See Reference.

Reference:

1. Biological Field and Laboratory Methods. 1972. EPA National Environmental Research Center, Analytical Quality Control Laboratory, Cincinnati, Ohio. (Preliminary Draft).

IN SITU PRODUCTIVITY OF PHYTOPLANKTON

Principal Detection Technique: Radioactive assay

Purpose of Measurement (Important Applications): Phytoplankton productivity measurements indicate the rate of uptake of inorganic carbon by phytoplankton during photosynthesis and are useful in determining the effects of pollutants and nutrients on the aquatic community.

Summary of Method: A solution of radioactive carbonate is added to light and dark bottles which have been filled with samples taken from preselected depths in the euphotic zone. Following *in situ* incubation (up to four hours) the plankton is collected on a membrane filter, dried in a desiccator, and assayed for radioactivity. The quantity of carbon fixed is proportional to the fraction of radioactive carbon assimilated. If measurements are required for the entire photoperiod, samples may be taken at overlapping four-hour periods or the data may be adjusted based on the solar radiation profile.

Limitations:

Range of Applicability: Phytoplankton

Pitfalls; Special Precautions: Carbon in the filtered sample should yield the number of counts required for statistical significance.

Statistical Characteristics:

Sensitivity: Method is more sensitive than the oxygen method, but fails to account for the organic materials that leach from cells during the incubation period.

Time of Measurement: Sample should be incubated for at least two hours.

Comments by Users: Method affords a direct measurement of carbon uptake and measures only photosynthesis.

Data Outputs: Instrument count

References:

1. Biological Field and Laboratory Methods. 1972. EPA National Environmental Research Center, Analytical Quality Control Laboratory, Cincinnati, Ohio. (Preliminary Draft).

2. Standard Methods for the Examination of Water and Wastewater. 1971. 13th Edition. American Public Health Association, Washington, D. C.

IN SITU PRODUCTIVITY OF PHYTOPLANKTON

Name and Measurement Method: Oxygen method

Purpose of Measurement (Important Applications): Phytoplankton productivity measurements indicate the rate of uptake of inorganic carbon by phytoplankton during photosynthesis and are useful in determining the effects of pollutants and nutrients on the aquatic community.

Summary of Method: Samples are taken from preselected depths in the euphotic zone and placed in duplicate clear, darkened and initial analysis bottles. The duplicate clear and darkened bottles are suspended at the depth from which the samples are taken and allowed to incubate. At the end of the exposure period the dissolved oxygen of the samples is measured. The increase in oxygen concentration in the light bottle during incubation represents net production and the loss of oxygen in the dark bottles is an estimate of respiration.

Limitations:

Range of Applicability: Phytoplankton

Pitfalls, Special Precautions: The incubation period should not be long enough to allow oxygen gas bubbles to form in the clear bottles or dissolved oxygen to be depleted in the dark bottles. The solar radiation profile in addition to the photosynthetic rate during the incubation period should be used to adjust data to represent productivity for the entire photoperiod.

Statistical Characteristics:

Time of Measurement: Samples should be incubated for at least two hours.

Comments by Users: Chief advantages of the oxygen method are that it provides estimates of gross and net productivity and respiration and that the analysis can be performed both with inexpensive laboratory equipment and common reagents.

Data Outputs: Visual observation (titrimetric) or analog or digital electrical signal (electrometric DO method).

Special Sampling Requirements (Collection, Storage, Handling): If the dissolved oxygen is not measured immediately, it should be fixed and protected from direct sunlight.

References:

1. Biological Field and Laboratory Methods. 1972. EPA National Environmental Research Center, Analytical Quality Control Laboratory, Cincinnati, Ohio. (Preliminary Draft).

PHYTOPLANKTON AND PERIPHYTON SPECIES COMPOSITION

Phytoplankton and Periphyton Species Composition

Principal Detection Technique: Visual examination

Purpose of Measurement (Important Applications): The identification of individual species of phytoplankton may provide information on indicator organisms, species diversity, and therefore the degree of pollution.

Summary of Method: Following an initial examination of the sample to obtain an estimate of population density, etc., the phytoplankton are identified to the desired taxonomic level and tallied under a standard system.

Limitations:

Range of Applicability: Plankton, periphyton

Pitfalls; Special Precautions: Examination is preferably done before sample is preserved. The beginner is strongly warned against the deceiving and nonvalid simplicity often found in the identification of plankton.

Statistical Characteristics:

Time of Measurement: 1 hour

Data Outputs: Number of species and the number of individuals per species

Special Sampling Requirements (Collection, Storage, Handling): In cases where it has been shown that preservation has no effects on the identification of organisms, a preservative should be used if storage is necessary.

References:

1. Biological Field and Laboratory Methods. 1972. EPA National Environmental Research Center, Analytical Quality Control Laboratory, Cincinnati, Ohio. (Preliminary Draft).

CHLOROPHYLL a, b, AND c OF PHYTOPLANKTON AND PERIPHYTON

Name of Measurement Method: In Vitro - Trichromatic Method

Principal Detection Technique: Spectrophotometric

Purpose of Measurement (Important Applications): All algae contain chlorophyll. Measurement of these pigments can yield an estimate of standing crop and taxonomic congestion.

Summary of Method: The sample is concentrated, macerated, steeped in 90% acetone, at 4°C for 24 hours, and clarified by centrifugation. The optical density of the decanted extract is determined at 750, 663, 645, and 630 nm using 90% acetone blank. The 750 nm reading is used to correct for turbidity and the other readings are inserted into SCOR/UNESCO equations for calculating chlorophyll a, b, and c.

Limitations:

Pitfalls; Special Precautions: Precautions should be taken to minimize evaporation. Pheophytin, a natural degradation product of chlorophyll, has an absorption peak in the same region of the visible spectrum as chlorophyll a and could be a source of error in chlorophyll determination.

Statistical Characteristics:

Calibration Requirements: No unusual requirements.

Data Outputs:

Data Outputs: Analog or digital electrical signal

Special Sampling Requirements (Collection, Storage, Handling): If the analysis will be delayed, store frozen. The stored samples must be kept in the dark to avoid photochemical breakdown of the chlorophyll. Maximum storage time is 30 days.

References:

1. Biological Field and Laboratory Methods. 1972. EPA National Environmental Research Center, Analytical Quality Control Laboratory, Cincinnati, Ohio. (Preliminary Draft).

CELL SURFACE AREA OF PHYTOPLANKTON

Principal Detection Technique: Visual observation

Purpose of Measurement (Important Applications): Determination of cell surface area of phytoplankton is another indicator of plankton abundance.

Summary of Method: Measure the dimension of several representative individuals of each major species microscopically. From the linear dimensions compute the average surface area per species. Multiply the area per species by the number of organisms per milliliter.

Limitations:

Range of Applicability: Phytoplankton.

Calibration Requirements: Ocular and stage micrometric

Data Outputs: Visual observation

Special Sampling Requirements (Collection, Storage, Handling): Depends upon the location and type of sample taken, see Reference.

References:

1. Biological Field and Laboratory Methods. 1972. EPA National Environmental Research Center, Analytical Quality Control Laboratory, Cincinnati, Ohio. (Preliminary Draft).

BIOMASS OF MACROPHYTES

Name of Measurement Method: Dryweight biomass

Principal Detection Techniques: Gravimetric

Purpose of Measurement (Important Applications): Estimates of growth rates related to pollution such as nutrient stimulation, can be accomplished by standing crop estimates at predetermined intervals.

Summary of Method: A sample is taken from a small defined area with conspicuous borders. The wet weight of material is obtained after the plants have drained for a period of time. The sample is then dried for 24 hours at 105°C and reweighed. The dry weight of vegetation per unit area is then calculated.

Data Outputs: Mechanical scale reading

Special Sampling Requirements (Collection, Storage, Handling): Before beginning a quantitative investigation it is desirable to have a statistical study design which will assist in determining the best sampling procedure, sampling area size, and number of samples.

References:

1. Biological Field and Laboratory Methods. 1972. EPA National Environmental Research Center, Analytical Quality Control Laboratory, Cincinnati, Ohio. (Preliminary Draft).

ALGAL GROWTH POTENTIAL

Name of Measurement: Bottle test method

Principal Detection Techniques: Volumetric, Gravimetric, etc. (depends on method used to indicate algal growth).

Purpose of Measurement (Important Applications): Algal assays are conducted to determine the effects of various discharges on the growth of algae or to determine whether or not various compounds or water samples are toxic or inhibitory to algae.

Summary of Method: After inoculation of test organisms the sample is incubated at 24 ± 2°C under cool white fluorescent lighting. During incubation, algal growth is determined by cell counting, or algal biomass or the chlorophyll analysis methods at predetermined intervals. The collected data are used to determine the maximum standing crop and the maximum specific growth rate.

Limitations:

Pitfalls, Special Precautions: When trace nutrients are being studied special galssware such as Vycor or polycarbonate containers should be used.

Statistical Characteristics:

Precision: Excellent agreement in data using this method was obtained by eight participating laboratories.

Time of Measurement: The analysis continued until there is less than 5 percent per day increase in biomass.

Calibration Requirements: See individual method in Reference cited below.

Data Outputs: Visual counts

Special Sampling Requirements (Collection, Storage, Handling): Sample pretreatment is required when determinations are being made of (1) growth limiting soluble nutrients or (2) amount of algal biomass then can be grown from all nutrients in waters.

References:

1. Biological Field and Laboratory Methods. 1972. EPA National Environmental Research Center, Analytical Quality Control Laboratory, Cincinnati, Ohio. (Preliminary Draft).

CHLOROPHYLL a OF PHYTOPLANKTON
(Fluorescence)

Name of Measurement Method: *In vivo* method

Purpose of Measurement (Important Applications): All algae contain chlorophyll *a*. Measurement of this pigment can yield an estimte of standing crop.

Limitations:

Range of Applicability: Scale deflection should be between 15 and 90 units.

Interferences: Phaeohytin

Pitfalls; Special Precautions: This method is less efficient than the extraction method, yielding about one-tenth as much fluorescence per unit weight as the same amount in solution.

Statistical Characteristics:

Sensitivity: Method is more sensitive than the spectrophotometer method.

Precision: The precision of the method using natural population shows for ten samples, a maximum variation of 15 percent.

Time of Measurement: Less than 2 hours.

Calibration Requirements: The fluorometer should be calibrated with a chlorophyll extract which has been analyzed with a spectrofluorometer.

Comments by Users:

Data Outputs: Analog or digital electrical signal

Special Sampling Requirements (Collection, Storage, Handling): Prolonged storage of extracts (greater than 5 days) under refrigeration and darkness should be avoided. Fluorescence of extracts kept at room temperatures and in light are stable for at least ten hours.

References:

1. Yentsch, C. S. and D. W. Menzel. 1963. A Method for the Determination of Phytoplankton Chlorophyll and Phaeophytin by Fluorescense. Deep-Sea Research 10: 221-281.

2. Biological Field and Laboratory Methods. 1972. EPA National Environmental Research Center, Analytical Quality Control Laboratory, Cincinnati, Ohio. (Preliminary Draft).

3. BIOASSAY PROCEDURES

3. BIOASSAYS

The purpose of this section is to discuss some available bioassay procedures for determining "safe" levels of pollutants. Bioassays are essential to evaluate a given pollutant in terms of existing water quality, including environmental variables as well as pollution already present. Pertinent to this stance is the fact that the majority of specific pollution problems are those involving discharge of unknown and variable composition. Almost without exception, more than one toxicant or stress is present. It is believed that "safe" levels of toxic pollutants, predicated upon bioassays with application factors, adequately protect aquatic life, if the basic physical parameters, e.g., dissolved oxygen, temperature, and pH are within the limits recommended. If the latter parameters are outside recommended limits, appropriate alterations in the criteria for toxicants must be made.

Acutely Lethal Versus "Safe" Concentrations

Harmful effects of pollutants may be divided into a number of categories which overlap somewhat; most could be described accurately by using one or two of the following terms, all of which have good dictionary meanings. In the present context of toxicity to aquatic life, the terms might be briefly defined as follows:

acute - involving a stimulus, severe enough to bring about a response speedily, usually within four days for fish;

subacute - involving a stimulus which is less severe than an acute stimulus, and which produces a response in a longer time; may become chronic

chronic - involving a stimulus which is lingering or continues for a long time; often signifying periods of about one-tenth of the life span or more;

lethal - causing death, or sufficient to cause it, by direct action;

sublethal - below the level which directly causes death;

cumulative - brought about, or increased in strength, by successive additions.

threshold - the point at which a physiological effect begins to be produced.

These and other terms have sometimes been used in careless or conflicting ways, but they should be used with precision. In general, two broad categories of effects may be distinguished; acute toxicity, which is usually lethal, and chronic toxicity, which may be lethal or sublethal (1).

Most of the available acute toxicity data are reported as the median tolerance limit (TLm) or median lethal concentration (LC50). Either symbol signifies the concentration at which 50 percent of the test organisms survive within a specified time span, usually in 96 hours. The customary 96-hour (four day) time period is recommended as adequate for most routine tests of acute toxicity with fish and the lethal threshold concentration attained within this time is usually reported (2). Mortality tests of a week or longer are necessary at times to determine the threshold concentrations. The lethal threshold concentration, as well as absence of any threshold concentration, should be reported. The lethal threshold concentration is more useful for comparative purposes than the arbitrary LC50.

This system of reporting is merely a convenient reference point for expressing the acute lethal toxicity of a given toxicant for the test animal. Usually, the "safe" levels (those which permit reproduction, growth, and all other normal life-processes in the fish's natural habitat) are much lower than the LC50.

Data on both long-term effects and safe levels are available for only a few toxicants. Some information is now becoming available on the effect of toxicants on reproduction, a very important aspect of all long-term toxicity tests. Also important is the sensitivity of the various life stages of organisms. Many organisms are most sensitive in the larval, nymphal, molting, or fry stage; some may be most sensitive in the egg and sperm stage.

It would be desirable if a single, universal, rapid, biological test could be used to directly measure sublethal effects of a pollutant. A large number of sublethal responses of fish have been used, i.e. specific physiological and biochemical changes in various body systems, and histological studies. A review of these shows that unfortunately, no single test is meaningful for all kinds of pollutants (3).

Variable Concentration

Criteria for continuously acceptable concentrations must be lower than the intermittent higher concentrations that may be reached occasionally but briefly without causing damage. One way in which to resolve this difficulty is to use both maximum short-time concentrations and also a more restrictive range of safe concentrations for continuous exposure. It is recognized that extremes do limit organisms, but, within these extremes there are ranges of concentration that can be tolerated, and are safe for prolonged periods of exposure. Most of the criteria recommended in Volume I of this report are those thought to be safe for continuous exposure.

In field situations and industrial operations, average 24-hour concentrations can be determined by using a small water pump to collect small aliquots of a few ml every few minutes, or to collect a slight trickle continuously. After 24 hours, the sample is mixed and analyzed and the concentration found represents the average concentration. Samples obtained this way are more reproducible and easier to secure than the maximum instantaneous concentration. But average concentrations are of little significance if fish are killed by a sharp peak of concentration; hence the need to determine maximum concentrations.

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METHODS OF BIOASSAY

The use of bioassays to determine the toxicity of a material or waste is the most effective and accurate method of predicting or assessing potential danger. In use of a bioassay, no assumptions need be made concerning the chemical structure or form of the pollutant, nor does the investigator have to know the constituent substances. The effects of water quality on toxicity also may be measured. of a toxicant in water, the more precise the assay can be.

Bioassays to Determine Acutely Lethal Concentrations

While there are many types of assays, two are in general use: the static bioassay in which the organisms are held in a tank of standing test-solution, which may or may not be changed during the period of the test; and the continuous flow or flow-through bioassay in which the test solution is renewed continually. Occasionally the difference between the two types is not very great, but sometimes one has clear advantages over the other.

A recommended outline of methods for routine bioassays is given by "Standard Methods" (4). Methods for more searching tests and for research purposes have been critically reviewed (3,5,6). The items which should be described in bioassay reports have been published (7,8).

The procedure for acute bioassay with fish is becoming standardized. Almost without exception, present-day tests incorporate:

1) a series of test containers, each with a different but constant concentration of the toxicant;

2) a group of similar fish, usually ten, in each container;

3) observations of fish mortality or other detrimental responses during exposures which last between 1 day and about 1 week, often four days; and

4) final results expressed as concentration tolerated by the median or "average" fish.

Beyond this, there are many other factors which make for good practice, and these are summarized in the references cited above. In essence, the items cited below serve as a checklist of good bioassay procedure.

Species

A fish or other aquatic organism of local importance should be used in bioassays. Preferably the test organism should be a game or panfish, since they are generally among the more sensitive species. Ability to duplicate an experiment would be enhanced if one used a selected strain of fish (9). A selected strain would also enable one to determine the difference between toxicants more reliably and to better determine the cause for variation in results due to different test apparatus. However, a selected strain does account for different sensitivities due to the response to other stresses such as disease, population stress, etc., which may be effecting a resident population.

Differences between fish species in susceptibility to a toxicant are generally less than might be expected - sometimes no greater than the variability within a single species tested in different types of water. For example, trout and carp are about equally resistant to some toxicants if tests continue for several days, giving the "coarse" species time to react (10,11). On the other hand, larvae and juveniles are most often more sensitive than adults and may be preferred for the test procedure. For protecting entire ecosystems, criteria suitable for the selected test fish will often also be suitable for other aquatic animals and plants. There are exceptions to this generalization. For example, copper is quite damaging to algae and mollusks, and insecticides are especially dangerous to aquatic arthropods. There are data to allow one to predict these situations. When they are expected, bioassays should also be run with invertebrates and algae (12).

Dilution Water

When possible, the bioassay should be conducted in waters obtained from the water body of concern since characteristics of the water, e.g., hardness, are included in the test results. Work of less immediate application, for example, when attempting to predict the toxicity of a new waste under various conditions, a selection of water types should be tried. there is considerable merit in using both hard and soft, synthetically prepared dilution waters (13) or varying the salinity of marine systems. There is great danger in using "tap water" for dilution, if it has been chlorinated. Even dechlorination procedures do not always remove the last traces of chlorine, and much of the past toxicity research is accordingly suspect.

Tap water can contain dangerous concentrations of copper, zinc or lead from plumbing.

Variations in other physical-chemical characteristics of water such as temperature, organic content and pH commonly affect toxicity of pollutants. Effects of five such environmental entities on the lethal threshold of ammonia were illustrated a decade ago (13, 14).

Water hardness is particularly important in toxicity of metals. Hydrogen ion concentration is a most important modifying factor for ammonia and cyanide. Higher temperatures sometimes increase toxicity of a pollutant, but recent work shows that phenol, hydrogen cyanide, ammonia, and zinc may be more toxic at low temperatures (15).

In static tests there should be two or three liters of water per gram of fish, changed daily, or increased proportionally in volume for the number of days of the test. In continuous flow tests, the flow must provide at least two or three liters of water per gram of fish per day, and it must equal test-volume in five hours, giving 90 percent replacement in half a day or less.

Acclimation

Acclimation of the test organism to the new environmental situation encountered before the bioassay begins may have a marked effect upon the outcome. Abrupt changes of quality of holding water should be avoided. Time for acclimation of the organisms to the new test conditions including temperature, should be as generous as possible and dependent on life-span, at least two weeks is recommended for fish.

Test Methods

These must be adequately described when the results are given and the easiest way is to follow one of the recommended bioassay procedures in Table 1.

Controls

Adequate and appropriate control tests must always be performed (2). Survival of control organisms is a minimum indication of the quality of test organisms. In addition, levels of survival and health in holding tanks should be indicated and the conclusions recorded.

TABLE 1 (CONT'D)

Kind of Organism	Type of Response	Appropriate Situations for Use	Reference
Fish	Respiratory movements as acute sublethal	Quick (1 day) indication of possible sublethal effects. Research and Monitoring.	19
Fish (*i.e.*, fathead minnows, brook trout, bluegill)	Reprodution, growth, and survival	Chronic tests for research on "safe" concentrations.	20-25
Daphnia	Survival & Reprodution	Rapid completion of chronic tests for testing special susceptibility of crustaceans	25
Diatoms	Survival, growth and reproduction	A sensitive, rapid, chronic test for research, prediction, or monitoring*	26

TABLE 1 (CONT'D)

Kind of Organism	Type of Response	Appropriate Situations for Use	Reference
Marine Crustacean Larvae Mollusks	Survival, growth and development through immature stages.	A sensitive, rapid, chronic test for research, prediction, or monitoring*	27

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* Requires an operator with some specialized biological training.

Stability of Test Environment

The supply of dilution water must be adequate to maintain constant test conditions. In both static and continuous-flow tests, a sufficiently large volume of test water must be used, and it must be replaced or replenished frequently. This is to provide oxygen for the organism, dilution of metabolic wastes, limit changes in temperature, pH, etc., and to compensate for degradation, volatilization, intake and sorption of the toxicant. In static tests, there should be 2 or 3 liters oxygen per gram of fish, changed daily or else the volume proportionally increased for the number of days of the test. In continuous-flow tests, the flow must: (a) provide at least 2 or 3 liters oxygen per gram of fish per day, and (b) flow must equal test-volume in five hours or less, giving 90% replacement in half a day or less.

The problem of maintaining dissolved oxygen concentrations suitable for aquatic life in the test chamber can be difficult. The suggestions above on test-volume and replacement times should provide for adequate oxygen in most cases. However, with some wastes, insufficient oxygen may be present in the test water because BOD or COD may consume much of the available dissolved oxygen. Aeration or oxygenation may degrade or remove the test material. Devices for maintaining satisfactory dissolved oxygen in static tests have been proposed and used with some degree of effectiveness, and have been described (28).

Periodic measurements of concentration of the toxicant should be made at least at the beginning and end of the bioassay. If it is not possible to measure the toxicant introduced, concentrations may be stated alone but it should be realized that actual concentrations in the water may become reduced.

In the flow-through type of bioassay, sometimes a large quantity of testwater is made up in a batch, and used gradually; or more usually, a device is used to add the toxicant to a flow of water and the mixture is discharged into the test container. One system is the dosing apparatus (29). Such a doser would be built for each test tank, and thus the concentration, or the kind of toxicant, can be modified at will in each individual tank. Other suitable individual dosers based on different principles are available (30-32).

Statistical Evaluation of Results

Percentage mortalities at the longer exposure time should be plotted on a vertical probit scale against concentrations of toxicant on a horizontal logarithmetic scale. The concentration which causes 50% mortality can be read and used as the LC50. The procedures can be improved by estimating the error of the LC50 using the simple nomograph procedures of Litchfield and Wilcoxon (33). Results can be improved by frequently inspecting mortality. The LC50's can be estimated (by the above procedures) for a series of exposure times such as 1, 2, 3, 6, 12, 24, 48, 72 and 96 hours. A toxicity curve of time versus LC_{50} could then be constructed on logarithmetic axes. The lethal threshold concentration could then be estimated in many cases (3, 5, 6) and this usually provides a more valid single number for description of acute toxicity than the arbitrary-time 96-hour LC50.

Test Organisms

For important bodies of water, there is a strong case for testing several kinds of aquatic organisms, not only fish. A comparative study of 20 pollutants on fish, snails, and diatoms found that no single kind of organism was most sensitive in all situations (10).

The short-term bioassay method for fish can also be used for many of the larger invertebrate animals. Few changes in method are necessary except those of size, and a much greater volume of test water and/or rate of flow in relation to weight of the animals, since their metabolic rate is higher on a weight basis.

Methods for Using Diatoms as Bioassay Organisms in the Laboratory

Algae and other microorganisms are useful for bioassays because a chronic test can actually be done in a week, since cycles of reproduction and growth occur in that time (34). It is important in selecting one to remember that a kinetic rather than a two-point measure of growth rate is necessary to avoid errors introduced by lag and recovery phenomena commonly encountered.

Application Factors

Short-term or acute toxicity tests do not indicate concentrations of a potential toxicant that are harmless under conditions of long-term exposure. It is desirable, therefore, to multiply the 96-hour values by factors, to estimate concentrations of the constituent in question that is safe in the receiving water. Such factors have been called application factors. An application factor is not a safety factor since it makes no extra allowance of safety for unknown factors. It is merely a fractional or decimal factor applied to a lethal or effective concentration in a short term test, in order to estimate the safe concentration as accurately as possible.

Ideally, an application factor should be experimentally determined for each pollutant. To do this, it would first be necessary to determine the lethal concentration of the constituent(s) in question according to the bioassay procedures outlined above. To obtain the application factor it would be necessary to determine the truly "safe" concentration of the same constituent(s) on the same species by thorough research on physiological, biochemical, and behavioral effects, and by studying growth, reproduction, and production in the laboratory and field. The safe-to-lethal ratio obtained can then be used in the future as an application factor, to work from measured LC50's of similar kinds of water, to predict the safe concentration. Such bioassays should be repeated at least monthly.

For example, if the 96 hour LC_{50} is 0.5 mg/l and the concentration of the constituent found to be safe is 0.01 mg/l, the ratio would be:

$$\frac{\text{Safe Concentration}}{\text{96-hour LC50}} = \frac{0.01}{0.50} = \frac{1}{50} = 0.02$$

In this instance, the safe-to-lethal ratio is 0.02, which can be used as an application factor in other situations. Then, in a given situation involving this constituent, the safe concentration in the receiving stream would be found by multiplying the four-day LC50 by 0.02.

This predictive procedure based on lethal concentrations is useful since the safe level of many pollutants is not known, because of the uncertainty about the toxicity of mixed pollutants because of the difference in sensitivity among fish and fish food organisms. The various considerations involved in developing application factors are discussed in the literature (25). Results of studies in which continuous exposure was used reveal that the safe-to-lethal ratio which permits spawning ranges over nearly two orders of magnitude (20-24). It is recognized that exposure in most natural situations will not be constant and that higher concentrations usually can be tolerated for short periods.

Lethal threshold concentrations, which may require more than 96-hour exposure, may be beneficially used to replace 96-hour LC50's in the above procedures, and there is a current trend to use such threshold concentrations (24).

Mixtures of Two or More Pollutants

For the lethal action of a mixture, the total toxicity may be estimated by expressing the actual concentration of each toxicant as a proportion of its lethal threshold concentration (= 96 hour LC_{50} usually), then adding together the resulting number for all the toxicants. If the total is 1.0 or greater, the mixture is predicted to be lethal.

The system of "adding up" different toxicants is based on the premise that their lethal actions are simply additive, one with the other. This simple rule has been found to govern the combined lethal action of many pairs and mixtures of quite dissimilar toxicants and is true for such diverse toxicants as copper and ammonia: and zinc and phenol in both laboratory (36-38) and field studies (39). The method of addition is useful and reasonably accurate for predicting thresholds of lethal effects in mixtures. Also, there are cases where the combined actions of two or more toxicants exceed the simple additive effects of the individual toxicants. This phenomenon is commonly referred to as synergism. No technique is known for predicting increased lethality of mixtures of toxicants acting synergistically. Conversely there is evidence of mixtures of two or more compounds having a lethal effect that is less than the single additive effects of the individual toxicants. This phenomenon is commonly referred to as antagonism. Techniques for predicting the lethality of mixtures of antagonistic consitituents also are not known.

Sublethal or chronic effects of mixtures are of great importance for water quality criteria. Sublethal concentrations of different toxicants should also be assumed to be additive in effect. Here again, it would be expected that for any given toxicant there would be some low concentration which would have no deleterious effect on an organism, and would not contribute any sublethal toxicity to a mixture. Research is extremely scanty on this topic.

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B. Classification of Waters 304(a) (2) C

Classification of waters, depending upon the suitability for the protection of human health and for the propagation of fish and aquatic life is categorized as follows:

1. Protection of Human Health

a. Acceptable: Waters which are suitable for contact recreation.

b. Treatable (Acceptable with treatment): Waters which, although not suitable for contact recreation, are acceptable for drinking water supply intake and can be treated to acceptable potable water meeting human health standards.

c. Unacceptable: Waters which contain substances such as hydrocarbons (grease, oils) and hazardous substances which cannot be removed and thereby render the water unsafe for recreation or drinking water intake.

2. Protection of Fish and Aquatic Life

a. Acceptable: Waters which are suitable for the propagation of the most important sensitive species of aquatic biota.

b. Unacceptable: Waters that contain concentrations of pollutants that will not sustain "the most important sensitive species" of aquatic biota.

V. Identification of Pollutants Suitable for Maximum Daily Loads

304(a)(2)D

All pollutants described by Volume I, Water Quality Criteria, are potentially suitable for maximum daily load restriction; however, the existence of Water Quality Standards is a prerequisite for making this determination. Only those pollutants which have a specific limiting value in the Standards or those pollutants whose effects are specifically limited in the Standards are suitable for maximum daily load.

*U.S. GOVERNMENT PRINTING OFFICE: 1984 546-315/217 1-3